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The Venue


کارگر شمالی
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انستیتو پاستور ایران
Pasteur Institute
کارگر جنوبی
خیابان پاستور
میدان پاستور
خیابان پاستور

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General Information

Conference Information

- Date: May 26 (Wednesday) –27 (Thursday), 2010
- Venue: Pasteur Institute, Tehran
- Official Language: English
- Organized by: Iranian Proteomics Society (IPS)

Registration

- Registration Hours

  26 (Wednesday) 7:30 - 8:30
  27 (Thursday) 7:30 - 8:30

- Registration Fee Includes
  - Access to Scientific Sessions
  - Access to Exhibition Area
  - Conference Package (Bag, Abstract book, etc.)
  - Launch and Breaks
  - Certificate of Attendance

Exhibition Area (Industrial & Scientific Exhibition)

- Posters should be mounted on the matching poster board with their poster presentation numbers and removed at the end of poster display hours. Any posters remaining after display hours will be discarded.
- Posters setting time is from 7:30 am till 8:30 am on Wednesday, May 26.
- Poster Display Hours: from 7:30 May 26 till 16:00 May 27.

Coffee Break

Daily coffee break will be served in Exhibition Area during the conference period.

Certificate of Attendance

A Certificate of Attendance is issued for general delegates for conference at the registration desk after 18 p.m.

Contact Address

Iranian Proteomics Society (IPS)
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Scientific Program

Wednesday, May 26, 2010

May 26, 2010, 7:30 – 8:30, Registration

May 26, 2010, 8:30 – 9:00, Opening Ceremony

8:30 – 8:35	**Holy Quran**
8:35 – 8:40	**Iranian National Anthem**
8:40 – 8:45	**Congress Chairman, B. Vaziri**
8:45 – 8:50	**President of Iranian Proteomics Society, G.H. Salekdeh**
8:50 – 9:00	**President of Pasteur Institute of Iran, S.M.H. Moddaresi**

May 26, 2010, 9:00-10:30

Chair Persons: *J. J. Calvete (Spain), Y-K. Paik (Korea), Hosseini Salekdeh (Iran)*	
9:00 – 9:30 **Y-K. Paik (Korea)**	**In Search of Novel Serologic HCC Biomarkers: From A Single Protein to Multiple Panel Proteins**
9:30 – 10:00 **J. J. Calvete (Spain)**	**Snake venomics, antivenomics, and venom phenotyping**
10:00-10:30 **K. Nakamura** *(Japan)*	**ER Membrane Proteomics**

10:30-11:00: Coffee Break, Exhibition & Poster reading

May 26, 2010, 11:00-12:40

Chair Persons: *C. Borchers (Canada), B. Vaziri (Iran), N. Nakamura (Japan)*	
11:00 – 11:30 **B. Vaziri** (*Iran)*	**Comparative Proteomics in targeting problems of infectious diseases**
11:30 – 12:00 **C. Borchers** *(Canada)*	**Combining protein chemistry and mass spectrometry for structural proteomics**
12:00-12:30 **H. Mehrani** *(Iran)*	**Plasma proteomic profile of sulfur mustard exposed lung diseases patients using protein fractionation and 2-DE**

12:30-14:00: Lunch

May 26, 2010, 14:00-16:00

Chair Persons: *C. Finnie (Denmark), M. Toorchi (Iran), N. Kazemipour (Iran)*	
14:00-14:30 **C. Finnie** *(Denmark)*	**Analysis of redox-related proteins in barley seed proteomes**
14:30 – 14:45 **M. Toorchi** *(Iran)*	**Proteomics approach for identifying osmotic-stress-related proteins in soybean roots**
14:45 – 15:00 **M. Sepehri** *(Iran)*	**A proteomic approach to decipher molecular mechanism of induced salt- tolerance in barley by *Piriformospora indica***
15:00-15:15 **N. Kazemipour** *(Iran)*	**Proteomic characterization of periplasmic fraction of the plant pathogenic bacterium *Erwinia chrysanthemi***
15:15-15:30 **B. Nakhoda** *(Iran)*	**Transcriptom and proteome analyses of wild type IR64 and two mutants with contrasting responses to salt stress under control and stress conditions during vegetative stage**
15:30-15:45 **A. Shahpiri** *(Iran)*	**Characterization of thioredoxin system in barley seeds: gene expression, protein profiles and interactions between isoforms of thioredoxin h and thioredoxin reductase**
15:45 – 16:00 **S. H. H. Moghaddam** *(Iran)*	**Identification of Small Heat Shock Proteins (sHSP) of Silkworm by 2D Electrophoresis and Mass Spectrometry**

16:00-16:30: Coffee Break, Exhibition & Poster reading

May 26, 2010, 16:30-18:00

Chair Persons: *R. Westermeier (Germany), H. Mehrani (Iran), M. Habibi-Rezaei (Iran)*	
16:30– 17:00 **Reiner Westermeier** *(Germany)*	**Breakthroughs in the Gel-based Proteomics Workflow**

17:00 – 17:15 **S. Fazeli** *(Iran)*	**Proteome analysis of central nervous system protein in EAE model of MS after post-transplantation recovery**
17:15-17:30 **Z. Mojtahedi** *(Iran)*	**Identification of autoantigens in breast cancer by two dimensional immunoblot**
17:30-17:45 **A.Ramezani** *(Iran)*	**Exploring the proteome of *Plasmodium falciparum* after challenging with the extract of *Prosopis julifolra***
17:45-18:00 **Z. Zamani** *(Iran)*	**Detection of colon cancer using HNMR spectroscopy on serum samples using pattern recognition**

Thursday, May 27, 2010

May 27, 2010 8:30-10:00

Chair Persons: *Christine Finnie* (*Denmark), Behrouz Vaziri (Iran), H. Hashempour (Iran)*	
8:30 – 8:45 **S. Keyvanshokooh** *(Iran)*	**The Status of Sturgeon Proteomics in Iran**
8:45-9:00 **Fatemeh Zandi** *(Iran)*	**Quantitative analysis of neuroblastoma proteome alterations in response to CVS and PV strains of Rabies virus: Towards solving the puzzle of Rabies pathogenesis**
9:00-9:15 **L. Pirhaji** *(Iran)*	**Mathematical modelling of protein-protein interaction network using information theory approach**
9:15-9:30 **H. Hashempour** *(Iran)*	**Nano Liquid Chromatography Fourier Transforms Mass Spectrometry for Analysis of Cyclotides in Viola ignobilis**
9:30-10:00 **C.Finnie** *(Demark)*	**Proteome analysis of the interaction between barley and the fungus *Fusarium graminearum***

10:00-10:30 Coffee Break, Exhibition & Poster reading

May 27, 2010 10:30-12:30

Chair Persons: *Young-Ki Paik (Korea), Hosseini Salekdeh (Iran)*	
10:30 – 11:00 **Y-K. Paik (***Korea)*	**SNP centric Human Proteome Project**

11:00 – 11:30 **Hosseini Salekdeh** *(Iran)*	**Iranian Human Proteome Project-perspective**
11:30-12:30	**Open Discussion**

12:30-14:00: Lunch

May 27, 2010, 14:00-15:30

Chair Persons: *R. Westermeier (Germany), B. Gharesi-Far (Iran)*	
14:00-14:30 **R. Westermeier** *(Germany)*	**Electrophoresis of difficult proteins: Analysis of basic, hydrophobic, membranous proteins, Blue Native PAGE**
14:30-14:45 **B. Gharesi-Fard** *(Iran)*	**Proteome differences in unexpected recurrent pregnancy loss (URPL) compared to normal placenta**
14:45-15:00 **F. Shamsi** *(Iran)*	**A Comparative Transcriptomics and Proteomics Analysis to discover molecular mechanisms of enhanced survival rate of human embryonic stem cell in the presence of a ROCK inhibitor**
15:00-15:15 **S. Tarighi** *(Iran)*	**Proteome analysis of PA4203, a newly described regulator of fitness and quorum sensing in *Pseudomonas aeruginosa***
15:15-15:30 **A.Fathi** *(Iran)*	**Proteome and Transcripteome Analyses of Human Embryonic Stem Cells Differentiated To Neural Cells**

15:30-16:00: Coffee Break, Exhibition & Poster reading

May 27, 2010, 16:00-16:30

Chair Persons: *J. J. Calvete (Spain), Christoph Borchers (Canada), H. Mehrani (Iran)*	
16:00 – 16:30 **J. J. Calvete** *(Spain)*	**Inferring evolutionary trends from venomic analyses**
16:30 – 17:00 **k. Nakamura** *(Japan)*	**Protein Chip Technology for Cancer Biomarker Discovery**
17:00–17:30	**Novel approaches for absolute and multiplex**

C.Borchers *(Canada)*	**quantitative proteomics suitable for clinical application**

May 27, 2010, 17:300-18:00, closing

17:30–18:00 Awards and closing remarks

Invited Lectures

Dr. Christoph Borchers

Dr. Borchers received his B.S., M.S. and Ph.D. from the University of Konstanz, Germany. After his post-doctoral training and employment as a staff scientist at NIEHS/NIH/RTP, NC and he was the director of the Duke – UNC Proteomics Facility and held a faculty position at UNC Medical School in Chapel Hill, NC (2001-2006). Since then Dr. Borchers is Associate Professor at University of Victoria (UVic), Canada and the Director of the UVic – Genome Proteomics Centre. His research is centred around the improvement, development and application of proteomics technologies with major focus on techniques for quantitative targeted proteomics for clinical diagnostics as well as for mass spectrometric based structural proteomics.

Prof. Juan J. Calvete

Dr. Juan J. Calvete is Research Professor of the Spanish Research Council (CSIC), Professor of PEDECIBA (Uruguay), and Head of the Structural Proteinomics Laboratory at the Instituto de Biomedicina de Valencia. He studied Biology at the University of Valencia, and earned his Ph.D. degree in Biochemistry from Complutense University (Madrid, 1985). He completed post-doctoral training in protein chemistry and structural biology at the Banting Institute (Toronto, Canada) (1987), the Max-Planck-Institute für Biochemie (Martinsried, Germany) (1988-92), and the Institut für Reproductionsmedizin (Hannover, Germany) (1993-98) prior to assuming his current position in the Instituto de Biomedicina de Valencia in 1998. Dr. Calvete has coauthored more than 230 scientific publications, which have received over 6000 citations. His research has focused on structure-function correlations of the human integrin, $\alpha_{IIb}\beta_3$, the platelet receptor for fibrinogen (for which he received a Young Investigator Award of the International Society on Thrombosis and Haemostasis, 1989); and on proteins involved in mammalian reproduction (particularly from the spermadhesin family, which was discovered in the 90´s by his PhD student Libia Sanz in Prof. Edda Töpfer-Petersen´s lab). Since his return to Spain, the Calvete´s lab has concentrated on structural and functional proteomics of snake venoms. They have developed proteomic tool ("venomics", "antivenomics", and "venom phenotyping") for exploring the evolution, composition, and biotechnological applications of venoms and toxins (eg. disintegrins). Juan J. Calvete has served as first President of the Spanish Proteomics Society (SEProt). Currently, he is member of the Congress & Communication Committe of the European Proteomics Association (EuPA), Editorial Adviser of the Biochemical Journal, Editorial Board Member of Toxicon, and Editor-in-Chief of the Journal of Proteomics.

Dr. Christine Finnie

Dr. Christine Finnie obtained her B.Sc. in Biochemistry from Bath University, U.K. (1993). Her Ph.D. studies of protein secretion by the plant symbiotic bacterium *Rhizobium leguminosarum* were conducted at the John Innes Institute, Norwich, U.K. After finishing the Ph.D, she moved in 1997 to the Royal Veterinary and Agricultural University in Denmark, for postdoctoral studies of the interaction between barley and the powdery mildew fungus. After this she spent four years at the Carlsberg Laboratory, Denmark, working on proteome analysis of barley seed development and germination. In 2004 she moved to the Technical University of Denmark to continue her work on plant proteomics and since 2007 has been Associate Professor in proteomics and protein chemistry with responsibility for the proteomics platform. Her research interests involve application of proteomics and protein biochemistry to key developmental and signalling processes in plants, including cereal seed development and germination, plasma membrane proteomics, plant-pathogen interactions, role of the thioredoxin system and redox regulation in plants, and correlation of proteomes with quality parameters.

Ghasem Hosseini Salekdeh, Ph.D

Dr. Salekdeh received his PhD in Genetics at International Rice Research Institute, Philippines in 2002. His thesis work was focused on the proteome response of rice to drought and salinity stresses. He returned to Iran as an assistant professor at Agricultural Biotechnology Research Institute of Iran (ABRII), Karaj where he established Stress Proteomics lab. In 2005, he started his collaboration with Department of Stem Cells at Royan Institute, Tehran. He is council member of the Asia Oceania Human Proteome Organization (AOHUPO) and member of HUPO education committee. He is also chair of AOHUPO Embryonic Stem Cell Membrane Proteome Initiative (AOHUPO ESC-MPI) steering committee. On a national level, Dr. Salekdeh is Cofounder of Iranian Proteomics Society and President elect of society from 2004. He is on a number of editorial boards, including Proteomics journal. He received several awards and honors including National Biotechnology Award (2007), best researcher award from Ministry of Agriculture (2008), Razi Medical Science award for advance technologies (2009), and the Khwarizmi International Award for fundamental research (2010), and Hadavi award from Iranian Academy of Medical Sciences (2010). He is coordinator of Human Y Chromosome Proteome Project, a national project endorsed by Human Proteome Organization (HUPO). He has published over 40 peer-reviewed international papers and has written a text book on Molecular Markers.

Dr. Hossein Mehrani

Dr. Hossein Mehrani is a teaching and research professor at the Baqiyatallah Medical Sciences University and Head of the proteomics laboratory at the Institute of Systems Biology. He studied at the Ottawa-Carleton Institute of Biochemistry in Canada and earned his Ph. D in Biochemistry (1993). He completed post doctoral training in protein kinase signalling at the NRC (Ottawa Canada 1994). His research is centred on toxicology of organophosphates compound on human health. The improvement, development and application of Clinical Biochemistry in the diagnosis of Human diseases are another area of his studies. Recently he has established a proteomics laboratory and concentrated on Human lung disease proteomics.

Prof. Kazuyuki Nakamura

Dr. Kazuyuki Nakamura, Professor and Chairman in Department of Biochemistry and Functional Proteomics, Yamaguchi University Graduate School of Medicine, is serving for the promotion of human proteomics as a HUPO Council member, a member of Education & Training (E&T) Committee, a co-chair of Human Disease Glycomics Proteome Initiative (HGPI), an advisory member of Human Kidney and Urine Proteome Project (HKUPP), an AOHUPO Vice President (E&T Mission), a member of Membrane Proteomics Initiative (MPI), and a Past President of Japan HUPO. He is active in E&T of young scientists in Asia not only as a Member of HUPO E&T Committee but also a President of Japanese Electrophoresis Society (JES). He organized a Joint Meeting of AOHUPO symposium and JES symposium for the Satellite Meeting of 20th IUBMB to promote E&T in Japan. He is interested in the discovery of biomarkers and therapeutic targets for HCV-related Hepatocellular Carcinoma and Pancreatic Cancer using two-dimensional gel electrophoresis combined with tandem mass spectrometry, and in the development of Cys-tag-Protein Chips for high through-put analyses of protein-protein interactions. His interest is shifting to Membrane Proteomics, Cancer Immunology and Immuno-Proteomics for the development of new diagnostic tools and non-invasive curative treatments of malignant tumors. He is also serving for the promotion of proteomics as an Associate Editor of Proteome Science, and an Editorial Board Member of Proteomics, Expert Review of Proteomics, and so on. His mission is to promote E&T of graduate students and young scientists being experts in the field of functional proteomics and clinical proteomics for understanding Molecular System of Life.

Prof. Young-Ki Paik

Prof. Young-Ki Paik is a biochemist and molecular biologist by training and has worked in the discipline of proteomics with emphasis on the cancer and metabolic disease for many years. He earned his Ph.D. from the University of Missouri-Columbia in Biochemistry with the studies on cholesterol metabolism. While Paik continued his work on disease metabolism in Yonsei University he has accomplished several landmark works including his outstanding contributions to elucidation of the later stage of cholesterol pathway from lanosterol (cited in Biochemistry Textbooks, Voet & Voet, 3rd Ed, 2004) and identification of ferritin light chain as a biomarker of hepatocellular carcinoma using proteomics and genomics tools and discovery of daumone (dauer-inducing pheromone), which regulates aging of *C. elegans* (Jeong et al., 2005, Nature, 433, 451-455). Paik has been co-inventors of numerous United State Patents and Korean Patents that deal with many innovative new technologies and drug development.

As director of the Yonsei Proteome Research Center (YPRC) (www.proteomix.org), the first proteome research institute established in Korea in 1999, and its sister center Biomedical Proteome Research Center (BPRC) established in 2003, he has demonstrated an outstanding leadership in managing diverse teams of research scientists on an international scale in handling many complex problems ranging from the research projects to daily operation of administrative works.

Paik is now president of Human Proteome Organization (HUPO, 2009-2010) after finishing off his duty as VP and Secretary General for 7 years from 2001 to 2008. Paik, along with Asian-Oceania colleagues, has initiated a creation of AOHUPO and he is also serving as president for this organization. He has also been serving as the Inaugural President of the Korean Human Proteome Organization (KHUPO, >900 members), which was established in July, 2001 as the first HUPO regional society. He has published more than 100 peer-reviewed international journal papers. Paik is senior editor of Proteomics journal and editorial board member of several other journals.

Dr. Behrouz Vaziri

Behrouz Vaziri, pharmD and immunologist, has focused on protein chemistry since 1997 when he had one year postdoc training on protein purification and characterization in a technology transfer program of biopharmaceuticals for Pasteur Institute of Iran (PII).
In 1999, he established the protein chemistry unit at Biotechnology Research Center of PII. He sat up and validated different Pharmacopeia-based analytical methods used in characterization of different biopharmaceuticals and now provide analytical services and consults to Iranian Biopharma industries and authorities as well.
From 2004, he has been involved in different proteomics-based research projects in different fields of infectious diseases. Neural dysfunction aroused by deathly Rabies virus is his major research activity in this concern. Now, in collaboration with various groups in PII he is involved in different research project using comparative proteomics analysis, from finding out the serum biomarkers of *H.pylori* related gastric cancer to drug resistant molecular targets in Malaria and lesihmania.

Dr. Reiner Westermeier

Dr. Reiner Westermeier works as a scientific marketing director at gelcompany, a novel company formed from ETC Elektrophorese-Technik, Tübingen, Germany, Fluorotechnics Ltd., Sydney, and The Gelcompany, San Francisco.
From 1976 to 1984 Ph.D. student and Post-Doc in the group of Angelika Görg at the Technical University Munich in Weihenstephan, Germany. 1984 to 1990 he was application specialist for electrophoretic separation methods at LKB Instrument GmbH and Pharmacia LKB Biotechnology. In 1991he co-founded ETC Electrophorese-Technik together with Dr. Hanspeter Schickle for the development of new electrophoresis methods, media, and equipment. 1996 to 2008 he continued as global scientific support manager for Proteomics at Pharmacia Biotech, Amersham Biosciences, and GE-Healthcare to give lectures, seminars, and courses on various methodologies used in Proteomics on a global basis.
Author of numerous publications, book chapters, and two books ("Electrophoresis in Practice", VCH Weinheim, fourth englishedition 2004 at WILEY-VCH; and together with Tom Naven and Hans-Rudolf Hoepker: “Proteomics in Practice”, WILEY-VCH Weinheim, second edition 2008).
The professional interests are methodical developments and improvements, as well as application developments and troubleshooting within the area of separation and evaluation techniques used in Proteomics.

IL-1

In Search of Novel Serologic HCC Biomarkers: From a Single Protein to Multiple Panel Proteins

Keun-Na, Kwang-Youl Kim, Sang-Yun Cho, and Young-Ki Paik

[1]Yonsei Proteome Research Center, Department of Biochemistry and Integrated OMICS for Biomedical Sciences, Yonsei University, Seoul, Korea.

Hepatocellular carcinoma (HCC) is a common cancer worldwide and accounts for nearly 40% of all cancers and ~90% of primary liver cancers in Southeast Asia. Although HCC has been the subject of considerable research interest, the associated prognosis and death rates have remained nearly constant, probably due to poor diagnosis. Using the multilectin affinity chromatography coupled with 2D-DIGE and 2D-LC-MS/MS systems, we have previously identified and partially validated human liver carboxylesterase 1 (hCE1), an N-linked glycoprotein, as potential serologic biomarker candidate for HCC, which was significantly down regulated in tumor tissues but highly induced in plasma of HCC patients. To extend our studies on the biological role for hCE1, we attempted to establish hCE1-overexpressing HepG2 cell line and analyze differentially expressed proteins. Among those differentially expressed proteins examined, some proteins (with ±1.5-fold increase) ($p < 0.05$) such as TrpRS and PDIA3, are known to have cellular pro- or anti-apoptotic functions as validated at the mRNA level. We were interested in examining whether hCE1 relates to cell proliferation using two stable, normal cell lines (Chang liver, WRL-68) and two cancer cell lines (HepG2, PLC/PRF/5) by testing the growth pattern of these cells transfected with a pCMV-hCE1 vector and continuously grown in the presence of G418. The cell count analysis showed that the population of normal cells increases, whereas that of cancer cells decreases over a 5-day culture period. We also confirmed that the expression levels of cytochrome **c** as a pro-apoptotic factor and 14-3-3 sigma isoform as a tumor-suppressor were increased, suggesting that hCE1 may be one of the major regulatory factors controlling normal cellular homeostasis, possibly as a carcinogenesis blocker.

In an attempt to identify the multiple panel proteins which might also be useful for diagnostic purpose, we employed multiple reaction monitoring (MRM) that can sensitively and selectively quantitate proteolytic peptides as surrogates for the corresponding intact proteins. To establish and optimize a robust, rapid and cost-efficient assay system for biomarker verification based on the scheduled MRM strategy (sMRM), synthesized and tested each synthetic SIS peptide representing panel proteins that have been identified as HCC biomarker in our laboratory. The amounts of all heavy synthetic peptides were analyzed using OPA and FMOC derivitization followed by reversed-phase UPLC-fluorescence detection, and this value was used for calculation of the exact amounts of synthetic peptides in samples. We successfully identified all predicted transitions from targeted native tryptic peptides and SIS in a single assay with the UPLC-sMRM system and minimum prefractionation conditions. We also used the mTRAQ® system, a non-isobaric amine labeling strategy, to create global reference standards for quantitative comparisons of clinical samples and a rapid UPLC-MRM assay. In comparing datasets from these two strategies, we found that the positive correlations between overlapping data and the mTRAQ strategy provide more opportunities to identify interesting proteins involved in HCC, which then can be used as new target candidates. We propose that the UPLC-sMRM assaying of mTRAQ®-labeled tryptic peptides can be an efficient method of choice for routine clinical assays for HCC plasma protein markers across large clinical sample sets. *This study was supported by a grant from the Korean Health 21 R&D Project, Ministry of Health, Welfare and Family Affairs, Republic of Korea, [A030003] and Korean Research WCU grant R31-2008-000-10086-0).*

IL–2

SNAKE VENOMICS, ANTIVENOMICS, AND VENOM PHENOTYPING: *MÉNAGE À TROIS* OF PROTEOMIC TOOLS AIMED AT UNDERSTANDING THE BIODIVERSITY OF VENOMS

Juan J. Calvete
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Envenoming constitutes a 'neglected tropical disease' mainly affecting young agricultural workers in low-income countries of Africa, Asia and Latin America, causing over 125.000 deaths yearly. The only effective treatment for systemic envenomation is the intravenous administration of an antivenom. Knowledge of the toxin composition of venoms could aid in the design of toxin-specific antibodies exhibiting greater specificity and effectiveness that conventional systems. To this end, the protein composition of the venoms of species of genera *Viperinae* (*Macrovipera*, *Cerastes*, *Echis*, *Bitis,* etc.) and *Crotalinae* (*Sistrurus*, *Crotalus*, *Agkistrodon, Bothrops, Bothriechis, Lachesis, Atropoides, Cerrophidion, Porthidium,* etc.) have been analyzed by RP-HPLC, N-terminal sequencing, MALDI-TOF MS, and in-gel tryptic digestion, peptide mass fingerprinting and CID-MS/MS. As expected from the rapid amino acid sequence divergence of venom proteins by accelerated evolution, with a few exceptions, the product ion spectra did not match to any known protein using the MASCOT. The CID-MS/MS spectra were manually interpreted and the deduced peptide ion sequences submitted to BLAST search. This approach allowed us to assign unambiguously all of the isolated venom fractions to known protein families. Our proteomic approach complement transcriptomic studies by showing the relative abundance of the proteins that are secreted into the venoms. Venom proteomes are composed of proteins belonging to only a few protein families, each venom showing distinct degree of complexity. Geographical, individual and ontogenic venom variations are also observed. Intraspecific ontogenetic, individual and geographical venom variability are highly relevant in snakebite pathology and therapeutics since envenoming results from the venom of a single snake. A robust knowledge of the phylogeny and the immunological crossreactivity of venoms may be also relevant for optimizing the use, and improving the effectiveness, of current antivenoms. This talk addresses the application of proteomic protocols developed in our laboratory ('*snake venomics*', '*antivenomics*', and '*venom phenotyping*') to investigate the composition and natural history of snake venoms, and the crossreactivity of antivenoms against homologous and heterologous venoms. This information is relevant for optimization of the clinical use of existing antivenoms by establishing their range of therapeutic application. Toxins from the same protein family present in venoms from snakes belonging to different genera often share antigenic determinants. This circumstance offers the possibility of defining the minimal set of venoms containing the epitopes necessary to generate novel therapeutic broad-range polyvalent antisera.

IL-3

ER Membrane Proteomics

Xiulian Zhang, Yasuhiro Kuramitsu, Masanori Fujimoto, Toshiyuki Tanaka, Junnko Akada, Hiroko Furumoto and Kazuyuki Nakamura
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Endoplasmic reticulum (ER) is a subcellular compartment playing a pivotal role in homeostasis of cellular functions. Beside its role in calcium storage and signaling, the ER lumen is the compartment where the protein folding of all membrane and secretary proteins takes place. In this study we carried out the protein profiling of ER fraction in Jurkat cells of a human T lymphoblastic leukemia cell line during heat stress. The ER fractions were prepared by differential centrifugation followed by sodium carbonate washing and treated with acetone methanol to yield delipidated ER proteins. The proteins were separated by SDS-PAGE into 58 protein bands which were visualized by CBB R-250 staining, and 128 proteins were identified by tandem mass spectrometry using nano-LC MS/MS. Tweleve protein bands including 60S ribosomal protein L4, L5, L15, L18, L19, L21, L26, L29, 40S ribosomal protein S5, S6, S18, and eukaryotic initiation factor 4A were found to be decreased by the heat stress incubating the cells at 45^0C for 30 min. Two protein bands of ubiquitinated proteins including eukaryotic translation initiation factor 3 were found to be increased by the heat stress. Further analyses by immunoblotting showed that phosphorylated-eIF2alpha of a maker of unfolded protein response (UPR) was increased by the heat stress. From these results, the heat stress might induce UPR and ER-associated degradation (ERAD) in Jurkat cells.

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IL-4

Comparative Proteomics in targeting problems of infectious diseases

Behrouz Vaziri
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Infectious diseases are in the world top 10 list of deaths. Lack of preventive vaccines and curative drug targets besides the puberty are the major causes of this high mortality rate. Moreover, the emergence of new infectious diseases and increased appearance of drug resistant pathogens make the situation more complicated.
Addressing these problems, comparative proteomics as a powerful tool could be used to target the biochemical pathways altered in host cells or to reveal the proteome changes in different strains of a given pathogen with various mode of activity.
In our laboratory, we have focused on pathogenicity and drug resistance mechanisms as well as predictive biomarker discovery using different proteomics approaches. In order to find the drug target candidate against deathly rabies we examined the viral pathogenicity mechanism by in vitro and in vivo models, and with various strains of rabies virus. Moreover, the biochemical pathways involved in drug resistant *Plasmodium falciparum* were investigated to find out the underlying cause of the phenomenon. In a different attempt, we have tried to find possible biomarker in sera of patients with *Helicobacter pylori* related gastric cancer.
Keywords: Infectious Disease, Proteomics

IL-5

Combining protein chemistry and mass spectrometry for structural proteomics

Christoph Borchers

One focus of the University of Victoria – Genome British Columbia Proteomics Centre is the development and application of mass spectrometry (MS) based approaches in structural proteomics. Here, we combine protein chemistry with MS, including H/D exchange and top-down MS using ECD, photoaffinity labeling/crosslinking and numerous MS techniques. Crosslinking combined with mass spectrometry is an emerging approach for studying protein structure and protein-protein interactions. Unambiguous mass spectrometric identification of crosslinked peptides derived from proteolytically digested crosslinked proteins is still challenging, however. At this point, we describe the use of novel chemical crosslinkers and novel crosslinking straegies, that promise to overcome many challenges associated with other crosslinking reagents and techniques. These crosslinkers are distinguished from others by a unique combination of properties including the capability of being isotopically-coded, fluorescent and cleavable either chemically or through dissociation in the mass spectrometer. Furthermore, some of the crosslinkers have affinity tags for selective enrichment of crosslinked peptides. Novel strategies are focused on the specific enrichment of inter-crosslinked peptides which are most informative for characterizing protein-protein interaction. Beyond proof-of-principle on model complexes the usefulness of these novel crosslinkers and strategies for biological samples are already shown. For example, using our fluorescent and photo-cleavable crosslinker BiPS we provided the first direct evidence describing the docking site of a phosphorylated G-protein coupled receptor C-terminus on the multi-functional adaptor protein β-arrestin. This clearly demonstrates the broad potential and application of these novel crosslinker in structural and cellular biology.

IL-6

Plasma proteomic profile of sulfur mustard exposed lung diseases patients using protein fractionation and 2-DE

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Sulfur mustard (2, 2-dichlorodiethyl sulfide, SM) is a chemical warfare agent that remains a threat to human health. The aim of the present study was to identify protein expression signature that reflect lung damage induced by SM exposure. The plasma albumin and immunoglobulins were depleted using aurum serum protein mini kit or plasma was fractionated using ethanol precipitation. Depleted or fractionated protein profile of ten healthy and thirty exposed patients with mild, moderate and severe lung diseases (10 males in each group) were separated with two dimensional SDS-PAGE and selected protein spots were successfully identified with MALDI TOF MS/MS. Results show that some haptoglobin isoforms were detected in all moderate and sever lung disease patients but none of the mild and healthy controls. Amyloid A1 isoforms was also detected in moderate and severs lung disease patients but none of the mild and healthy controls. Compared to the depletion method, the ethanol fractionation showed superior results both in protein spots numbers and intensity. Our present results and previous studies suggest that ongoing tissue remodeling is involved in sever lung damage and plasma level of haptoglobin and serum Amyloid A1 isoforms may be a potential biomarker in SM exposed lung diseases patients.

Keywords: Amyloid A1, haptoglobin isoforms, plasma proteomics, ethanol fractionation

IL-7

Analysis of redox-related proteins in barley seed proteomes

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Protein profiles of barley seed tissues are analysed during grain filling, maturation, germination and radicle elongation, and several hundred barley seed proteins have been identified [1]. Thioredoxins are protein disulphide reductases that regulate the intracellular redox environment and processes including DNA synthesis, oxidative stress responses and apoptosis. The ascorbate-glutathione cycle plays a central role in maintainance of cellular redox balance and is likely to include interactions with the thioredoxin system. Functional proteomics approaches focusing on the seed ascorbate-glutathione cycle and thioredoxin system will be described. Identification of potential thioredoxin target proteins and structural studies provide insight into determinants for target protein recognition by thioredoxin [2].

This work is supported by the Danish Centre for Advanced Food Studies, the Danish Research Council for Natural Science, the Danish Research Council for Technology and Production Sciences and the Iranian Ministry for Science, Research and Technology

[1] Finnie C, Svensson B (2009) Barley seed proteomics from spots to structures. J Proteomics 72: 315-324

[2] Shahpiri A, Svensson B, Finnie C (2009) From proteomics to structural studies of cytosolic/ mitochondrial type thioredoxin systems in barley seeds. Molecular Plant 2: 390-406

IL–8

Breakthroughs in the Gel-based Proteomics Workflow

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Proteome analysis presents many challenges to the researcher including the complexity of the samples, the large size and hydrophobicity of many proteins, the detection and characterization of post-translational modifications and large dynamic range of protein expression. To address these challenges a number of different methodology have been developed: Of these 2-D gel based workflow have been the most widely adopted and have the most general applicability. However, 2D-based methods have been increasingly criticized as being difficult, unreliable and poorly reproducible. New methods for 2D-gel based analysis are becoming available that, when combined, make the running of gels straightforward, reliable and reproducible.

Many of problems associated with 2D-gel based methods can be solved by DIGE (difference gel electrophoresis), where proteins of different biological samples are tagged with different fluorophores prior to the separation. By co-running the samples in the same 2-D gel and scanning the fluorophores at different wavelengths a multiplex result is obtained. When an internal standard, containing aliquots of each sample, is run in each gel, gel-to-gel variations are eliminated. Gel image analysis is then performed automatically with specially developed software.

A new generation of HPE (high performance electrophoresis) pre-cast gels which are on plastic backings has recently become available that are simple to use and provide reproducible results. Using these gels on a multi-level flatbed instrument, resolution, sensitivity, and the number of spots to be identified with mass spectrometry markedly increase.

New data obtained using DIGE and other fluorescent stains in combination with the novel HPE system will be shown.

IL-9

Proteome analysis of the interaction between barley and the fungus *Fusarium graminearum*

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Fusarium head blight (FHB) or scab, caused by *Fusarium* species including *Fusarium graminearum* in humid and semihumid climates, is a devastating disease in barley and other cereals. The disease reduces the grain yield due to floret sterility as well as poor grain filling and reduced kernel size. In addition to deceased yield and quality, the infected grains often contain mycotoxins which are hazardous to animals and humans. Various studies suggest that the type and amount of nitrogen fertilizer can affect the incidence and severity of FHB, however with contrasting results. An investigation is presented of the combined effect of N fertilisation and FHB on the barley seed proteome, providing molecular insight into the effect of N on FHB infection of barley [1]. One effect of FHB is degradation of plant proteins. Measurements of fungal biomass and fungal-induced proteolysis allowed proteome and gene expression analysis at a well-defined stage of the interaction, prior to extensive proteolysis. Discrete proteolytic fragments were identified which can serve as a proteome-level indicator of infection and grain protein quality.

This work is supported by the Danish Directorate for Food, Fisheries and Agri Business (DFFE), Plant Biotech Denmark, and the Centre for Advanced Food Studies (LMC).

[1]Yang F, Jensen JD, Spliid NH, Svensson B, Jacobsen S, Jørgensen LN, Jørgensen HJL, Collinge DB, Finnie C (2010). Investigation of the effect of nitrogen on severity of Fusarium head blight in barley. J Proteomics 73: 743-752

IL-10

cSNP-Centric Human Proteome Project

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A single-nucleotide polymorphism (SNP) is a DNA sequence variation occurring when a single nucleotide — A, T, C, or G — in the genome differs between members of a species (or between paired chromosomes in an individual). One nucleotide change could lead to creation of new splicing variant, alteration in enhancer, promoter and homeobox sequences, truncation of protein size and removal of post-translational modification site (e.g., UCG (Ser) to UUG (Leu)). Among those known SNPs, nonsynonymous SNP (nsSNP or cSNP) which usually leads to creation of missense (changing amino acid sequence) or nonsense (making stop-codon), becomes interested in human proteome project (HPP). It is predicted that cSNP would profoundly affect on pathogenesis of disease and response to pathogens, chemicals, drugs, vaccines, and other agents. Thus, cSNP may be key factor in establishing the concept of personalized medicine that is relying on genomics and proteomics technologies. In this talk, I will touch on several key issues as to utilization cSNP in HPP in the context of technological hurdles (e.g., reproducibility of peptide detections, distinction between heterozygote/homozygote), use of SRM/MRM for target proteins and sample sizes by exemplifying some cases in the study of hepatocellular carcinoma. *This study was supported by a grant from the Korean Health 21 R&D Project, Ministry of Health, Welfare and Family Affairs, Republic of Korea, [A030003] and Korean Research WCU grant R31-2008-000-10086-0).*

IL-11

Iranian Human Proteome Project-perspective

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The complete sequencing of the human genome gives scientists access to unprecedented numbers of gene-encoded proteins which are the "building blocks of life" and "cellular machinery". In the post-genomic era, there is an increasing need to decipher the temporal and spatial functions and interactions of proteins under different physiological conditions, developmental stages, and in response to environmental cues. However, it is estimated that for nearly half of the proteins encoded in the human genome, there is no experimental evidence for their protein existence and for many others there is very little information related to protein abundance, localization, and function. A gene-centric human proteome project will facilitate the defining of proteomic landscape in the human body and the discovery of new diagnostic and therapeutic tools and will stimulate biological and medical research. In Iran, the current efforts are focused on mapping the proteome of human chromosome Y, a project endorsed by Human Proteome Organization (HUPO). The Y chromosome is unique under many aspects. It is always in the haploid state and full of repeated sequences but it is responsible for important biological roles such as sex determination and male fertility. The goal of human Y chromosome Proteome Project (YHPP) is to fill the void between genotype and phenotype for basic science discovery and clinical application. A YHPP consists of antibody-based visualization of the major anatomical sites of protein expression with quantitation provided by antibody and mass spectrometry based innovations and protein interaction network resolved with various techniques. The integration of these efforts using bioinformatics will provide a resource for basic science and clinical communities. The technical and biological issues that need to be overcome will also be discussed.

IL-12

Electrophoresis of difficult proteins: Analysis of basic, hydrophobic, membranous proteins, Blue Native PAGE

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There are a number of challenges in proteome analysis, which should be met by the methodology employed: The complexity of the sample, the wide dynamic range of protein expression levels, high molecular weight and hydrophobicity of some proteins, the difficulty to detect and characterize post-translational modifications. Additionally, some proteins are particularly difficult to analyze: often because of a high hydrophobicity, high molecular weight, and a basic isoelectric point. Therefore sample preparation and running conditions for 2-D electrophoresis need to be optimized, in some cases the complex protein mixture must be pre-fractionated.

Very hydrophobic proteins, like membranous proteins get mostly lost during conventional 2-D electrophoresis, applying isoelectric focusing combined with SDS PAGE. Alternative separation methods like acid gel electrophoresis in presence of a cationic detergent or blue native PAGE can increase the representation of such difficult proteins considerably.

IL-13

Inferring evolutionary trends from venomic analyses, or learning from evolution to fight the neglected pathology of snakebite envenomation

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The presence of a venom-secreting oral gland is a shared derived character of the advanced (Caenophidia) snakes. Given the central role that diet has played in the adaptive radiation of snakes, venom thus represents a key adaptation that has played an important role in the diversification of these animals. Intraspecific venom variation represents a well documented phenomenon since more than 70 years, and is particularly notorious among species that have a wide distribution range, highlighting the concept that these species should be considered as a group of metapopulations. Typification of geographic-associated venom phenotypes may render valuable molecular markers for taxonomical as well as medical purposes. *B. asper* and *B. atrox* are highly adaptable and widely distributed species, and ontogenetic and geographical variability in their venom composition and pharmacological profile have been reported. A comparative analysis of the proteomes and the immunoreactivity profile of *B. atrox* specimens from Venezuela, Colombia, Brazil, Perú, and Ecuador evidenced the existence of two geographically differentiated venom phenotypes and suggested the ocurrence of paedomorphism in the diversification of *B. atrox* venoms. Achieving sexual maturity while maintaining increased hemotoxic and lethal venom activities may have conferred evolutionary fitness to the ancestors of Amazonian *B. atrox*, thus fueling the paedomorphic trend. Another comparative venomic and antivenomic characterization of the venoms of newborn and adult specimens of the Central American rattlesnake, *Crotalus simus*, and of the subspecies *cumanensis*, *durissus*, *ruruima*, and *terrificus* of South American *Crotalus durissus*, suggested that the South American taxa have retained juvenile venom characteristics in the adult form (paedomorphism) along their North-South stepping-stone dispersal. The driving force behind paedomorphism is often competition or predation pressure. The increased concentration of neurotoxins crotoxin and crotamine in South American rattlesnake venoms strongly argue that the gain of neurotoxicity and lethal venom activities to mammals may have represented the key axis along which overall venom toxicity has evolved during *Crotalus durissus* invasion of South America. The paedomorphic trend is supported by a decreasing LNC (lethal neurotoxicity coefficient, defined as the ratio between the average LD_{50} of the venom and the crotoxin + crotamine concentration) along the North-South axis, coincident with the evolutionary dispersal pattern of the Neotropical rattlesnakes. The identification of evolutionary trends among Bothrops and tropical Crotalus, may have an impact in defining the mixture of venoms for immunization to produce an effective pan-American antivenoms.

IL-14

Protein Chip Technology for Cancer Biomarker Discovery

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A protein tag consisting of five tandem cysteine repeates (Cys-tag) at C-termini of proteins has been developed for immobilization of the proteins onto the surface of a maleimide-modified, diamond-like carbon-coated silicon chip substrate (Cys-tag-Protein Chip) (1). The technology of Cys-tag-Protein Chip can be used for discovery of biomarkers and therapeutic targets of diseases with a high sensitivity and reproducibility. The Cys-tag-Protein Chip using proteins of HSP70, Mn-SOD and Peroxiredoxin, which were detected to be candidates for tissue biomarker of hepatitis C virus-related hepatocellular carcinoma (HCV-HCC) by PROTEOMEX (2), was used for discovery of auto-antibodies in sera of patients bearing HCV-HCC with a high specificity and sensitivity. The Chip technology can be used not only for serum biomarker discovery of diseases such as cancer but also large scale analysis of auto-antigens in auto-immune diseases.

References:
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(2) Kuramitsu, Y. and Nakamura, K. Exp. Rev. Proteomics 2005, 2, 589-601.

Figure 1. Working Flow of Cys-tag-Protein Chip for Cancer Biomarker Discovery.

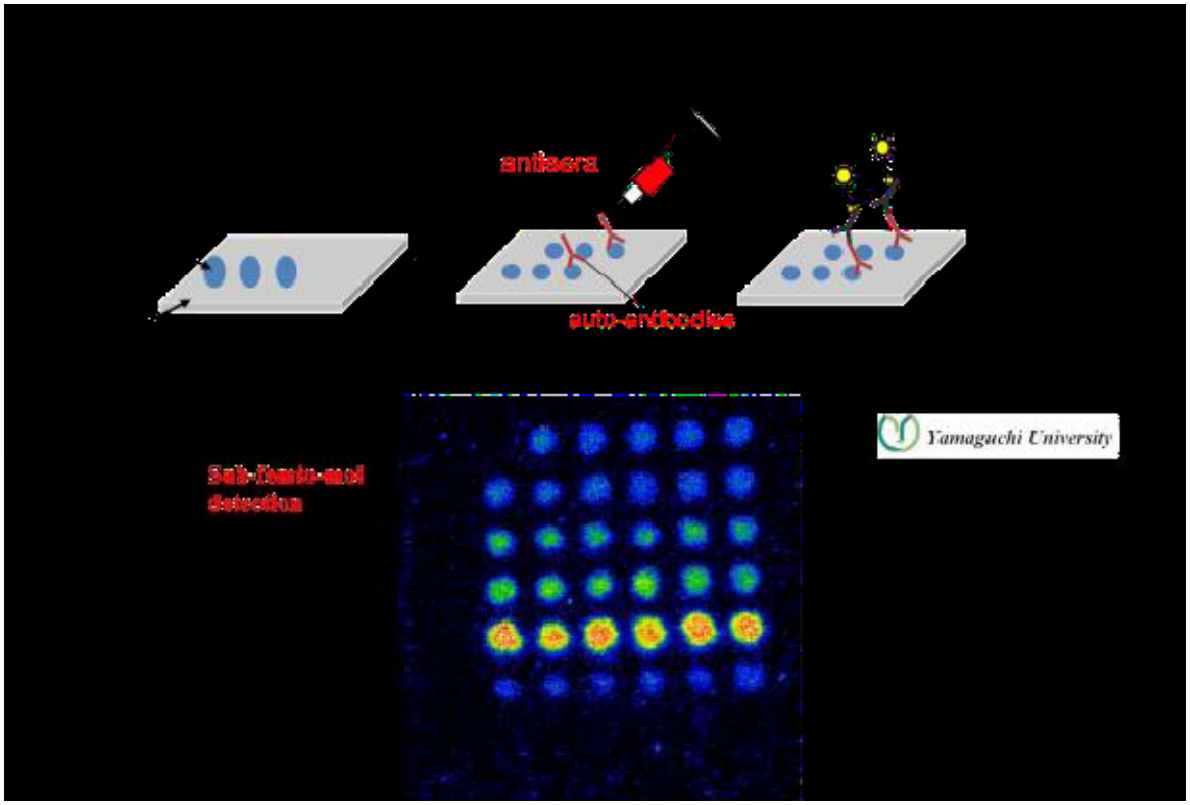

Figure 1. Working Flow of Cys-tag-Protein Chip for Cancer Biomarker Discovery.

IL-15

Novel approaches for absolute and multiplex quantitative proteomics suitable for clinical application

Christoph Borchers

One focus of the University of Victoria – Genome British Columbia Proteomics Centre is the development and application of mass spectrometry (MS) based proteomics approaches towards clinical implication. In particular, we are focused on further development and improvement of two MS centric approaches: Multi-Reaction Monitoring (MRM) and immuno-MALDI (iMALDI) – both have great potential for biomarker validation and discovery and translation into clinical set ups since these approaches are rapid, highly specific and enable absolute and multiplex protein quantitation. We have developed a 75 protein MRM-assay for validation of numerous cardio-vascular disease (CVD) biomarkers in human blood plasma and developed iMALDI approaches for the clinic. We applied the MRM-assay in a medium scale project analyzing 60 blood samples in triplicate verifying five proteins that are distinguishable between different CVDs. The iMALDI technology is a combined approach of immuno-enrichment of peptides followed by MALDI-MS (iMALDI). An iMALDI assay has been developed into a clinical assay for hypertension which will replace the currently used radio-immunoassay in a Vancouver hospital due to its higher specificity, speed, accuracy and sensitivity at lower cost.

Oral Presentations

OP-1

Proteomics approach for identifying osmotic-stress-related proteins in soybean roots

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Osmotic stress can endanger the survival of plants. To investigate the mechanisms by which plants respond to osmotic stress, protein profiles from soybean plants treated with polyethylene glycol (PEG) were monitored by a proteomics approach. Treatment with 10% aqueous PEG reduced the lengths of roots and hypocotyls of soybean seedlings. Proteins from soybean roots were separated by two-dimensional polyacrylamide gel electrophoresis, and 415 proteins were detected by Coomassie brilliant blue staining. Expression levels of 37 proteins were changed by PEG treatment, and this group included proteins involved in disease/defense, emphasizing the range of changes associated with dynamic osmotic signaling. The rapid induction of 9 proteins selected for time-course and dose-dependent expression analysis in PEG treatment indicated that they have signaling functions in mediating the osmotic response at the proteome level. A comparison with the effects of other abiotic stresses showed that Caffeoyl-CoA-o-methyltransferase and 20S proteasome alpha subunit A were decreased and increased by abiotic stresses, respectively. Expression analyses of these transcripts were also changed by PEG treatment. Caffeoyl-CoA-o-methyltransferase and 20S proteasome alpha subunit A may control the sensitivity of several regulatory genes specific to short exposure to osmotic stress.

OP-2

A proteomic approach to decipher molecular mechanism of induced salt tolerance in barley by *Piriformospora indica*

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Salinity is one the major limiting factors of crop production worldwide. Microbial endophytes, as one the most important soil microorganisms, can increase plant yield under biotic and abiotic stresses without modifying the genetic composition of plant. In this study, we investigated the molecular mechanisms of improved salt tolerance of barley (*Hordeum vulgare* L.) plants infected by *piriformospora indica*. Our results showed that *P. indica* significantly increased dry matter of infected barley under salt stress. We applied a proteomics approach to analyze leaf blade and sheath proteome of inoculated and non-inoculated plants under normal and salinity conditions. We observed that *P. indica* helped the inoculated plants to cope with stress by a coordinated expression of photosynthetic, metabolic and oxidative stress defense proteins. We extended our study by macroarray analysis of similar set of sample which resulted in identification of novel proteins. The combined results of proteomics and macroarray analyses will be discussed.
Key words: *Piriformospora indica*, Proteomics, Salt Stress, Barley.

OP-3

Proteomic characterization of periplasmic fraction of the plant pathogenic bacterium *Erwinia chrysanthemi*

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Erwinia chrysanthemi is phytipathogenic entrobacterium responsible for the soft-rot disease in a wide range of plants during growth, transit, and storage. Its pathogenic properties are mostly due to its ability to produce and secrete enzymes which are able to degrade constituents of the plant cell wall.
The recent availability of the sequence of the genome of the *E. chrysanthemi* strain 3937 allowed us to use proteomics to identify new proteins that could be involved in the pathogenicity of the bacterium. In the present study, we have identified the proteins present in the periplasm of *E. chrysanthemi* in the absence or presence of inducers of pectinase synthesis. We analyzed the periplasmic proteins, using two- dimensional electrophoresis and identified the proteins by analysis of mutant, western blotting and MALDI-TOF. Thirty four unique proteins were identified in this compartment, some of which were differentially expressed under the above condition. We found mostly proteins involved in active transport of substrates either identified, or predicated by sequence homology, or totally unknown. The inducible proteins are mainly involved in pectin catabolism or in iron assimilation, two essential factors of *E. chrysanthemi* virulence.
Keywords: Proteomics, periplasmic fraction, *Erwinia chrysanthemi*

OP-4

Transcriptom and proteome analyses of wild type IR64 and two mutants with contrasting responses to salt stress under control and stress conditions during vegetative stage

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Salinity is one of the major environmental constraints to rice production. Understating the logical relationships between gene and protein networks and their interactions with each other in generating salt responses under salinity stress is crucial for finding responsive genes for salinity tolerance. The contrasting mutants provide an effective system to study differential gene and protein expressions associated with salinity tolerance because of their high similarity in growth habit and phenology but different responses to salt stress. In this study we used genome-wide transcriptional analysis and a comparative two dimensional gel electrophoresis analysis to analyze gene expression in two contrasting mutants and their wild type parent. Salt stress imposed two weeks after planting and EC of the nutrient solution was gradually increased and adjusted at 12 dSm^{-1}. Shoot samples were collected for RNA and protein extraction at six days after reaching the final EC of 12 dS m^{-1}. Out of 850 proteins analyzed in rice short, we detected 78 protein spots with significant responses to salt stress. These proteins were involved in oxidative stress defense, metabolisms, photosynthesis, protein synthesis and processing, and signal transduction. Comparative analysis of IR64 mutants with their wild type parent, showed several proteins as key participants in salt stress tolerance.

We also analyzed similar set of samples using microarray analysis which resulted in identification of several novel mechanisms. Differentially expressed genes are located on different chromosomes showing that mutations are either affecting certain transcription factors that are regulating a larger set of genes and pathways or are affecting different loci throughout the genome. The possible role of identified mechanisms and genes in salt tolerance will be discussed.

Key Words: Rice, Salt stress, Transcriptional analysis, Microarray, proteomics

OP-5

Characterization of thioredoxin system in barley seeds: gene expression, protein profiles and interactions between isoforms of thioredoxin h and thioredoxin reductase

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Thioredoxins (Trx) are ubiquitous proteins that participate in thiol disulfide reactions via two active site cysteine residues, allowing Trx to reduce disulfide bonds in target proteins. Plants contain different types of thioredoxin systems. Chloroplast thioredoxins are reduced by a ferredoxin-thioredoxin reductase (FTR), whereas the cytosolic h-type thioredoxins are reduced by NADPH in a reaction catalyzed by NADPH-dependent thioredoxin reductase (NTR). Here we describe the identification, cloning, characterization and the first comparison of two NADPH-dependent thioredoxin reductase isoforms (HvNTR1 and HvNTR2) from barley. Their gene expression and protein profiles were analyzed in parallel with the two thioredoxin h isoforms (HvTrxh1 and HvTrxh2) in barley seed tissues. The results support a functional role of the NTR/Trx system during germination. Production of the recombinant proteins in *E. coli* as His-tagged proteins allowed the study of first *in vitro* reciprocal interactions between NTR and Trx h isoforms from the same species. This first investigation of regulation and interactions between members of the NTR/Trx system in barley seed tissues suggests that different isoforms are differentially regulated but may have overlapping roles, with HvNTR2 and HvTrxh1 being the predominant isoforms in aleurone layer.

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OP-6

Identification of Small Heat Shock Proteins (sHSP) of Silkworm by 2D Electrophoresis and Mass Spectrometry

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Probably silkworm as a domesticated insect is the most sensitive insect to heat. Therefore understanding more about thermal tolerance and heat shock proteins (HSPs) in the silkworm provides valuable information in both silkworm breeding and scientific aspects. Four breeds of silkworm, *Bombyx mori* L., were selected including Jingsong, a Chinese bivoltine breed, Haoyue, a Japanese bivoltine breed (both as heat-susceptible breeds), Nistari, a non-diapause polyvoltine as heat-tolerant breeds and P50, a diapause polyvoltine breed. There were four groups for each breed (two treatments and two sexes) which exposed to heat shock at 45°C for 30 minutes and 41°C for 1 hour (fifth instar, day 4) in a controlled growth chamber.
There were 16 specific response spots including 9, 4 and 3 in Nistari, Jingsong and Haoyue respectively and 5 common response spots belonged to low molecular weight area of SDS-PAGE of fat body proteom of each breed. P50 didn't express any specific response spots after heat exposure. Six out of 25 detected proteins in the proteome profiles were identified as HSPs by MALDI-TOF/TOF mass spectrometer in which 4 sHSP from commonly expressed proteins and 2 sHSP from specifically expressed proteins. While HSP19.9, HSP20.4, HSP20.8 and HSP23.7 were commonly expressed among all breeds but HSP20.1 and HSP21.4 were in Jingsong and Nistari respectively. Spot 2 from commonly expressed proteins obviously is a sHSP but PMF and MS/MS analysis could not match it with any sHSP in all the reapted experiments. It showed limitations of mass spectrometers to identify all differentially expressed proteins.

The discovery of variation in sHSP expression among silkworm breeds gives enough reasons to clarify in the future experiments the specific roles of each one of small heat shock proteins family in induced thermotolerance of silkworm larvae.

Keywords: Small heat shock proteins, proteomics, mass apectrometry, 2D electrophoresis

OP-7

Mouse embryonic stem cell-derived neural precursors in the treatment of EAE model of multiple sclerosis: a proteomics approach for discovering post-transplantation recovery mechanisms

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Multiple sclerosis (MS) is considered a chronic inflammatory and progressive disorder of the central nervous system (CNS), which ultimately leads to demyelination and subsequent axonal injury. Experimental autoimmune encephalomyelitis (EAE) is a well characterized animal models for studying the ethiology, pathogenesis and for testing new therapies of MS.

In this study, EAE was induced by myelin oligodendrocyte glycoprotein (MOG) in C57BL/6 mice. Then, EAE model at score 3 was treated with embryonic stem cell-derived neural precursor Morphological analysis showed recovery of EAE model of MS after transplantation. We sampled brains and spinal cords of mouse before and after recovery and studied their proteome using a two dimensional gel electrophoresis approach followed by identification of differentially expressed proteins using mass spectrometry. The results showed that the expression level of 13 brain protein spots 17 spinal cord protein spots of EAE mouse returned to normal level after post-transplantation recovery. These proteins belong to various functional groups including disturbance in ionic and neurotransmitter release, mitochondrial and energy metabolism, impairment of blood barriers, apoptosis, cytoskeleton proteins and signal transduction. The possible link of these proteins with MS will be discussed. Our results provide a proteomics view on the role of stem cell transplantation in MS treatment.

Keywords: Multiple sclerosis, EAE, Stem cell, Proteomics, Transplant

OP-8

Identification of autoantigens in breast cancer by two dimensional immunoblot

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Background: Endeavors to identify breast cancer-associated antigens by highthroughput techniques are being increased. These antigens may be useful in cancer diagnosis, prognosis, and immunotherapy. The aim of our study was to identify antigens eliciting a humoral immune response in breast cancer by a two-dimensional polyacrylamide gel electrophoresis, Western blotting, and mass spectrometry. **Methods:** Sera from HER2 positive and HER2 negative breast cancer patients and healthy volunteers were individually investigated for antibodies against MCF7 lysate. Reactive protein spots in immunoblots were matched to the stained gels. Matched spots were excised from the gels and subjected to MALDI-TOF/TOF MS analysis. **Results:** Some of the identified antigenic proteins were glycer aldehyde-3-phosphate dehydrogenase, adenosine kinase, fructose-bisphosphate aldolase, heat shock protein 27 and several other heat shock proteins. **Conclusion:** This study further strengths the usefulness of an immunoproteome analysis in the successful identification of immunoreactive proteins.

Keywords Breast cancer, Two Dimensional Gel Electrophoresis, Immunoproteome, MCF7.

OP-9

Exploring the proteome of *Plasmodium falciparum* after treatment with the extract of *Prosopis julifolra*

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Increasing drug resistance by *Plasmodium falciparum*, the most virulent of human malaria parasites, is creating new challenges in malaria chemotherapy. In the screening of antimalarial property of some Iranian plants we found that *Prosopis juliflora* had potent activity against malaria parasite *P. falciparum in vitro* (IC_{50}=1.4 µg/ml) and was also active *in vivo* against *P. berghei* in BALB/c mice (100 mg/kg body weight) .To find some new potential drug targets in *P. falciparum* proteome, we treated the culture of chroquine-resistant (K1) and –sensitive (F32) strain of *P. falciparum* with dichloromethane fraction of *Pr. juliflora* herb extract. After culture synchrozization to ring stage by sorbitol, the culture treated with herb extract after 28 h post-infection in trophozoite stage. After parasite release from red blood cells the whole protein extracts were undergone 2-DE and mass spectrometry analysis. Our findings indicated that the extract of *Pr. juliflora* affected the expression of proteins that were involved in protein folding, energy metaboilsm, cell adhesion, DNA repair, RNA recognition and some host proteins.

Keywords: *Plasmodium falciparum*, *Prosopis juliflora*, Drug target, Proteomics

OP-10

Detection of colon cancer using [1]HNMR spectroscopy on serum samples using pattern recognition

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Colon cancer is on the increase worldwide in both males and females, especially in economic transitional countries including Eastern European and most parts of Asia, and some countries in South America. Preventive strategies are occult fecal blood test, CEA tumor marker identification, both of which are relatively non specific and the specific but highly invasive colonoscopy. The increasing availability of high-throughput methodologies opens up new possibilities for more specific early screening. new markers. There has been no report about diagnosis of colon cancer using serum samples with [1]HNMR spectroscopy.

This study was carried out on 20 colon cancer patients which were on a liquid diet for 48 hours and were ready for colonscopy. 4 ml of blood was collected in heparinized tubes on ice and centrifuged to separate their plasma in less than 30 minutes. 800 µl of separated plasma was diluted with 10% D2O and [1]HNMR spectroscopy was carried out using CPMG spin echo. The spectrum obtained was fourier transformed and metabolites detected by using Mestrec Nova software. The results were then analyzed by principle component analysis using MATLAB software. A very good separation was obtained with glycine, β glucose, fucose, D-galactose and creatinine as the primary metabolites responsible for these separations. The amount of fucose and D-galactose were higher in serum samples of colon cancer patients than those of normal.

OP-11

The Status of Sturgeon Proteomics in Iran

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Proteomic studies are increasingly being used in fish biology and aquaculture research. To date, only a few reports have been published on sturgeon proteomes focusing on reproductive biology and ecotoxicology. However, the shortage of previous genetic data on most sturgeon species has been a main weakness for current proteomic studies on sturgeon species. This review describes proteomic approaches that have been used to investigate various biological subjects in sturgeon species in Iran.
Key words: Aquaculture, Sturgeon, Proteomics

OP-12

Quantitative analysis of neuroblastoma proteome alterations in response to CVS and PV strains of Rabies virus: Towards solving the puzzle of Rabies pathogenesis

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Following our previous attempts to solve the puzzle of the neuronal dysfunction induced by rabies infection, a murine neuroblastoma cell line was infected with challenge virus standard (CVS) and attenuated (PV) strains of rabies virus and the dynamic cellular proteome responses were determined at 48 h post infection using 2DE and MALDI TOF/TOF analysis. Comparative analysis of gels representing whole cell extracts from control, PV and CVS infected neuroblastoma cells revealed a total of 67 quantitatively altered protein spots (p<0.05). Of these, 41 spots (belonging to 25 proteins) were identified by MS analysis, which were categorized as viral protein (phosphoprotein isoforms) and host proteins involving in regulation, energy synthesis, protein biosynthesis, signal transduction, metabolism, anti-oxidative stress processes, and cytoskeleton structure. Changes in selected candidate proteins were verified by western blot analysis. Most significant expressional changes were found for CVS infected cell proteome, compared to PV infected cells and this could be related to difference in pathogenicity of mentioned strains of virus. Among expressed viral proteins, eight phosphoprotein isoforms were detected in CVS infected cells, while nothing was observed as viral phosphoprotein in PV infected cells. The most expressional changes of host proteins due to CVS infection were related to cytoskeletal network proteins (such as vimentin) and regulatory proteins of that network. Therefore reorganization of vimentin and disruption of cellular architecture during infection could be important for productive infection. In conclusion this study provides some important insights into host cell-rabies virus interaction at a molecular level which could be used for better understanding of rabies virus pathogenesis and accelerating antiviral research.

Keywords: Rabies, Neuroblastoma, proteomics, pathogenesis

OP-13

Mathematical modeling of protein-protein interaction network using information theory approach

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Understanding and modeling of cellular processes depend on comprehensive information of protein interaction networks. Large-scale affinity purification coupled with mass spectrometry (AP-MS) provided comprehensive data for the analysis of protein complexes. In large-scale AP-MS experiment, there are many different conditions in which different proteins are tagged, and in each pull-down there is high number of proteins which include a lot of contaminants. So dealing with this large amount of data to infer a reliable protein-protein interaction network is an essential task. Here, we propose a new algorithm which uses the concept of information theory for analyzing the parallel proteomic data. Information-theoretic methods use mutual information, which is an information-theoretic measure of dependency. Mutual information is being used for calculating the association score of each protein interaction based on measuring the similarity of protein profiles among different pull-downs. So with this algorithm we will be able to infer protein-protein interaction network with weighted edges using quantitative mass spectrometry, in which the weight of each interaction indicate the probability of the occurrence of that interaction.

OP-14

Nano Liquid Chromatography Fourier Transforms Mass Spectrometry for Analysis of Cyclotides in *Viola ignobilis*

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The cyclotides are macrocyclic plant peptides with three disulfide bounds. The backbone of cyclotides cyclized head-to-tail made unique structure, topology and stability [1]. These peptides typically comprise about 28-37 amino acids and are the greatest group of known circular proteins family [2]. These plant peptides have shown interest resistance to enzymatic degradation, high temperature and various pH conditions [3]. Cyclotides are playing as host defense function in plant against insecticides, pesticides and pathogens [4]. A wide variety of biological activities for cyclotides have reported include cytotoxic [5], anti-microbial [6], anti-HIV [7] and haemolytic activity [8].

In this work, a nano liquid chromatography fourier transform mass spectrometry (nano-LC FTMS) technique has been used for analyses of cyclotides in *viola ignobilis*. The cyclotides from *viola ignobilis* were collected from east Azerbaijan of Iran and extracted by dichloromethane/methanol (1:1, v/v). Then, the sample was dried and a C18 solid phase extraction (SPE) with ethanol: water (50:50) was used for sample preparation. According to nano-LC FTMS results, a view of cyclotides profiles of a plant could be obtained. The disulfide bounds of these peptides were reduced by chemical reactions (iodoacetamidate) and were re-analysed for confirmation of their structures. Four known and two unknown cyclotides were determined in viola *ignobilis*.

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OP-15

PROTEOME DIFFERENCES IN UNEXPLAINED RECURRENT PREGNANCY LOSS (URPL) COMPARED TO NORMAL PLACENTA

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Introduction & aim: Recurrent pregnancy loss (RPL) is defined as at least two sequential abortion before the 20th week of gestation. In approximately 40% of RPL cases the etiological factors which causes abortion are unknown and so named, unexplained RPL (URPL). Placenta is a pregnancy unique tissue and proper formation of the placenta is a key phenomenal for success a pregnancy or occurrence of UPRL. Therefore, the aim of the present study is comparison of the human placental proteome between URPL and normal first trimester placentas.

Material & methods: Total placental proteins were extracted and two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) technique was used to compare the proteome of five URPL and five gestational matched normal placentas. After staining, the gels were scanned and the spots intensities were determined using Image Master 2D Platinum Software and compared between URPL and normal cases. Statistically differentially expressed spots were excised from the gels and identified by Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF/TOF) technique after in gel digestion.

Results: Though 19 spots showed statistically different expression ($p<0.05$), 12 out of them were successfully identified. Among them only two proteins were down-regulated (Calumenin, Enolase 1) while the remaining ten spots (Actin gamma 1 propeptide, Cathepsin D prepropeptide, HSPgp96, Tubtlin bet, Tubulin alpha 1, Glutathione S-transferase, vitamin D binding protein, Prohibitin, Actin beta, Apolipoprotein A-I) showed increased expression in URPL cases in comparison with normal first trimester placentas.

Conclusion: In conclusion, the data of the present study indicated that the alteration in expression of proteins involves in endothelial dysfunction might play an important role in the pathogenesis of URPL.

OP-16

A Comparative Transcriptomics and Proteomics Analysis to discover molecular mechanisms of enhanced survival rate of human embryonic stem cell in the presence of a ROCK inhibitor

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Human Embryonic stem cells (hESCs) have the potential to differentiate to all types of cells in the human body and hold a great promise for application in regenerative medicine. Improvement of hESCs culture conditions can accelerate their clinical applications. Recently, it was shown that treatment of dissociated hESCs with Y-27632 (ROCKi), a potent inhibitor of Rho-kinase, could increase survival rate of hESCs. We applied a two-dimensional gel electrophoresis approach to compare the proteome profiles of two hESCs lines in the absence or presence of ROCKi in the medium and extracellular matrix (Matrigel). More than 1100 protein spots were compared on 2-D gels of each hESC line. Student *t*-test ($p \leq 0.05$) showed that expression level of 50 protein spots were up or down-regulated by 1.5-folds after Y-27632 treatment. Of them only 39 protein spots were detected on preparative gels and identified by MALDI-TOF-TOF mass spectrometry. We analyzed similar set of samples using a microarray approach to identify the main signaling pathways activated or inhibited in hESCs in response to ROCKi. Most of the differentially expressed genes at the mRNA or protein levels were clustered into six functional groups including: metabolic processes, cytoskeleton organization, regulation of developmental processes, cell-cell adhesion, cell proliferation, and apoptosis. The possible implications of these mechanisms in enhanced undifferentiated growth of hESCs will be discussed.

OP-17

Proteome analysis of PA4203, a newly described regulator of fitness and quorum sensing in *Pseudomonas aeruginosa*

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In *Pseudomonas aeruginosa* the PA4203 gene, upstream of *ppgL*, encodes a LysR-type regulator similar to BenM and CatM in *Acinetobacter spp*. We described that the *Pseudomonas aeruginosa ppgL* (PA4204) gene, which encodes a protein with a signal peptide and with a COG2706 domain corresponding to a 3-carboxy-cis, cis-muconate lactonizing enzyme, could affect the fitness of *P. aeruginosa*. Cis,cis muconate is the effector for BenM and CatM, suggesting that the product of PA4204 could be the effector for PA4203 and could be a molecule similar to cis,cis muconate. Interestingly, a deletion of PA4203 affected the level of expression of *ppgL* and *mexGHI-opmD*. Furthermorethe PA4203 gene was highly expressed in a PA4204 mutant. This suggests that the product of PA4204 is the effector of the LysR regulator, which negatively regulates its own transcription. The PA4203 mutant grew like the wild-type, but did not produce the blue-green pigment pyocyanin on Pseudomonas P agar. Surprisingly, the same mutant was completely unable to produce the siderophore pyoverdine in CAA medium, but the production of the second siderophore, pyochelin, was not affected. The PA4203 mutant was hypomotile and also more sensitive to H_2O_2 compared to that of wild-type. Proteome analysis was done using 2D-PAGE with different staining methods (coomassie blue, silver staining, and DIGE). Cellular protein extracts of the PA4203 showed a low expression of some outer membrane proteins (OmpA) and extracellular chitinase. On the other hand, production of trigger factor, flagellin type B, the two component response regulator PhoP/Q and 50S ribosomal protein L9 were more pronounced in the PA4203 mutant. Secretome analysis showed that the production of two different types of the flagellar capping protein FliD and FliG in the PA4203 mutant versus WT, while the production of the chitin-binding protein CbpD precursor present in WT was not observed in the PA4203 mutant. The above results suggest that the LysR type regulator encoded by PA4203 is needed for the expression of *ppgL* (which is a modulator of quorum sensing) and *mexGHI-opmD* (which are part of the PQS regulon) and for the production of virulence factors.

OP-18

Proteome and Transcripteome Analyses of Human Embryonic Stem Cells Differentiated To Neural Cells

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Human embryonic stem cells (hESCs) are pluripotent cells capable of differentiating into other cell lineages in vivo. Study of Molecular events in hESCs differentiation to neural cells will help to further define molecular mechanisms involved in the neutralization and neuronal fate determination and differentiation. We employed a 2-D DIGE based proteomics approach to analyze hESC line, Royan H6, in undifferentiated cells, neural ectoderm, neural rosette and mature neurons after differentiation. The expression of 137 spots modulated during differentiation, of them 118 differentiation associated proteins could be identified using MALDI-TOF/TOF and LC MS/MS. These include several proteins involved in apoptosis, cell cycle, cell structure and motility, protein and nucleotide metabolism, translation, mRNA processing and protein folding. We further analyzed these samples at mRNA level using whole genome microarray chip. Functional analyses of regulated genes support morphological state of the cells. Owing to comparative proteomics and transcriptomics analyses, novel genes involved in proliferation of rosette cells, fate specification and neural differentiation were identified.

Keyword: Proteomics/ microarray/Embryonic stem cells/ neural cells/ Human

Posters

P-1

Proteome study of sera from chronic hepatitis, cirrhosis and hepatocellular carcinoma patients related to hepatitis C virus by 2-DE-LC-MS/MS

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Introduction and aim: Hepatocellular carcinoma (HCC) is the fifth most common and third leading cause of cancer dead worldwide. Infection with HCV is a major cause of chronic hepatitis, cirrhosis and HCC around the world and approximately 30% of HCC cases are related to this virus. Due to poor sensitivity and specificity of available diagnosis tools, diagnosis of HCC is usually occurred at an advance stage, and accordingly, the 5-year survival of HCC patients is only 5-7%. So, early diagnosis remains the key to effective therapy for survival improvement of the patients.

Aim: The aim of this study was to identify proteins and/or protein patterns differentially expressed in HCC patients compare to cirrhosis and chronic hepatitis related to HCV infection by two dimensional polyacrylamid gel electrophoresis (2D- PAGE) and liquid Chromatography Mass Spectrometry (LC-MS/MS).

Materials and methods: Sera from patients with chronic hepatitis, cirrhosis and HCC patients related to HCV were subjected to 2D- PAGE. After silver staining, gels were scanned with GS-800 scanner and analyzed by prodigy proteomics analysis program. Differentially expressed proteins were excised from gels, destaied and determined by LC-MS/MS.

Results: Twenty nine protein spots were differentially expressed between chronic hepatitis and cirrhosis patients. Thirteen protein spots were different between HCC and chronic patients and 9 spots were differentially expressed between cirrhosis and HCC patients more than 1.5 fold. Identifying proteins include different isomers of haptoglobin, clusterin, transthyrtin and zinc α glycoprotein.

Conclusion: These data show that 2-DE-LC-MS/MS is a useful tool for serm biomarker discovery. These differentially expressed proteins maybe useful for early, easy and cost benefit diagnosis of cirrhosis and HCC patients related to HCV.

Key words: Hepatocellular carcinoma, Hepatitis C virus, Proteome, LC-MS/MS

P-2

Proteome study of sera from cirrhosis and hepatocellular carcinoma patients related to hepatitis B virus by 2-dimentional electrophoresis and liquid chromatography mass spectrometry

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Introduction: Hepatocellular carcinoma (HCC) is the fifth most common cause of cancer and the third cancer- killer worldwide. More than 50% of HCC cases are related to hepatitis B virus (HBV) infection. The 5-year survival of HCC patients is only 5% that in part related to the diagnosis of HCC at an advance stage, because of poor sensitivity and specificity of available diagnostic tools. So, early diagnosis remains the key to effective therapy for survival improvement of the patients.

Aim : The aim of this study was to identify proteins and/or protein patterns differentially expressed i in HCC patients compare to cirrhosis hepatitis related to HBV infection by two dimensional polyacrylamid gel electrophoresis (2D- PAGE) and liquid chromatography mass spectrometry (LC-MS/MS).

Materials and methods: Fourteen Sera from cirrhosis and HCC patients related to HBV were subjected to -2D- PAGE. After silver staining, gels were scanned with GS-800 scanner and analyzed by prodigy software for finding differentially expressed proteins. Differentially expressed proteins picked up from gels, destined and some of them were determined by LC-MS/MS.

Results: We found that cirrhosis and HCC patients had different serum protein patterns. Thirty two spots were differentially expressed between cirrhosis and HCC patients more than 1.5 fold including haptoglobin αchain and clusterin.

Conclusion: Our data show that sera from cirrhosis and HCC stage of hepatitis have different protein pattern. These proteins maybe useful to include in surveillance program of cirrhosis patients for early diagnosis of HCC and/or related to the hepatocarcinogenesis of HBV virus.

Key words: Hepatocellular carcinoma, Hepatitis C virus, Proteome, LC-MS/MS

P-3

Proteome study of sera from hepatocellular carcinoma patients related to hepatitis B virus and hepatitis C virus by 2-DE-LC-MS/MS

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Introduction and aim: Hepatocellular carcinoma (HCC) is the fifth most common cause of cancer and the third cancer- killer worldwide. Hepatitis B virus HBV and hepatitis C virus HCV are the most common cause of HCC. Although HBV and HCV can be cause HCC in long time but they are different viruses and use different mechanisms for liver carcinogenesis.
Aim: The aim of this study was to identify proteins that are differentially expressed in sera from HCC patients related to HBV and HCV by two dimensional polyacrylamid gel electrophoresis (2D- PAGE) and liquid chromatography mass spectrometry(LC-MS/MS).
Materials and methods: Twelve Sera from HCC patients related to HBV and HCV were subjected to -2D- PAGE. After silver staining, gels were scanned with GS-800 scanner and analyzed by prodigy software for finding differentially expressed proteins. Differentially expressed proteins picked up from gels, destained and were identified by LC-MS/MS.
Results: We found that HCC patients related to HBV and HCV had different serum protein patterns. Twenty spots were differentially expressed between two groups more than 1.5 fold, including two isomers of haptoglobin alpha chain, 3 isomers of leucine- rich alpha glycoprotein and paroxanase.
Conclusion: Our data show that sera from HCC patients related to HBV and HCV have different protein patterns. These differentially expressed proteins maybe related to different mechanisms which involve in hepatocarcinogeneses. So our date maybe reveal different hepatocarcinogenesis mechanisms between hepatitis B and C viruses.

Key words: Hepatocellular carcinoma, Proteome, 2-DE- LC-MS/MS

P-4

Proteome analyss of freeze tolerant winter wheat during transition from the vegetative to the reproductive phase under field condition

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Phase transition from vegetative to reproductive growth is an important character for winter wheat in cold regions because it decreases the ability of wheat plants to maintain frost tolerance. Using proteomic analysis, an investigation aimed at a better understanding of the proteins alternation during the vegetative/reproductive phase transition was carried out in hardy winter wheat (*Triticum aestivum* L., cv. Norstar) under field condition in cold region (Zanjan). Proteins were extracted from the leaves, collected from plants during three different growth stages including T1: vegetative growth when plants increased their frost tolerance (23-Nov, LT_{50}: -12 ºC), T2: vernalization saturation point when plants acquired to maximum frost tolerance (21-Dec, LT_{50}: -28 ºC) and T3: after vegetative/reproductive Phase transition (21-Feb, LT_{50}: -16 ºC). Change in protein expression was determined by two-dimensional (2-D) gel electrophoresis. Approximately 400 protein spots were reproducibility separated and visualized on CBB-stained 2-D gels. 110 protein spots were found to be up-regulated in response to transition from T1 to T2 stage and 34 proteins had been down-regulated. Spots comparison between T3 and T2 revealed that 160 spots were responsive which 76% had been up-regulated. Expression profile of some identified proteins like as cell division control protein, asparagine synthase, translational elongation factor, ribulose-1,5-bisphosphate carboxylase activase, actin, harpin binding protein and heat shock protein 70kDa were similar to LT tolerance trend during the investigated stags.

P-5

Proteome analysis of two contrasting rice mutants and wild type parent under control and salt stress conditions at the vegetative stage

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Salinity is a major limiting factor in rice (*Oryza sativa* L.) production worldwide. It is increasingly important in irrigated environments as a result of water quality and poor maintenance of infrastructure. Rice is among the most sensitive crops to salinity, especially at early vegetative stage. We used proteomics technique to study differential protein expression in rice mutants with contrasting responses to salt stress. Two selected IR64 mutant lines with better tolerance or higher sensitivity to salt stress compare to their wild type parent, IR64, were planted under controlled conditions (29/21°C day/night temperature and 70% RH) in hydroponics using Yoshida nutrient solution. Salt stress imposed two weeks after planting and EC of the nutrient solution was gradually increased and adjusted at 12 dS m^{-1}. Shoot samples were collected for protein extraction at six days after reaching the final EC of 12 dS m^{-1}. The profile of differentially expressed proteins was studied using total protein extracted from plants grown under control and salt stress conditions. SES scores, Na^+ and K^+ concentrations in shoots and Na^+/K^+ ratio were significantly different in contrasting mutants under salt stress conditions. A comparative two dimensional gel electrophoresis (2-DE) analysis on rice shoot was applied. Out of 850 protein spots reproducibly detected, 78 protein spots showed significant response to salt stress. We used mass spectrometry (MALDI TOF/TOF) analysis to identify the protein spots. The MALDI TOF/TOF analysis detected all the 78 proteins. These proteins were involved in various molecular processes including oxidative stress defense, metabolisms, photosynthesis, protein synthesis and processing, signal transduction. Owing to a comparative analysis of IR64 mutants with altered responses to salt stress and their wild type parent, several proteins emerged as key participants is salt stress tolerance. Possible roles of these genes in plant adaptation to salt stress will be discussed.

Keywords: Rice, Mutants, Salt Stress, Proteomics.

P-6

Proteomics Study of Silver Nanoparticle - Peptidoglycan Cell Wall Interaction

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In this study the effects of synthesized silver nanoparticles (SNPs) on the bacterial cell wall were investigated. The influence on the peptide branches was considered as well as the glycan strands. To evaluate any structural variations the *Staphylococcus aureus* peptidoglycan cell wall, as gram positive model bacteria, were chosen. In order to study the peptide secondary structure mutation the circular dichroisms (CD) was performed. On the other hand the primary structure variations not only were studied using high performance liquid chromatography-mass spectrometry (LC-MS), but also compared with the result of the tryptic digestion. With respect to the results, the SNPs not only changes the secondary structure (*α-helix*) of bacterial cell wall, but also destroyed its primary structure. These results indicate that the reaction with SNPs affects the peptide primary structure and increasing the number of degradation products after these PGNs treated with SNP.

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P-7

Applications of proteomics in dentistry and dental tissue engineering

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Proteomics research has already provided some insights into normal biology and human disease through. Our teeth are highly sophisticated structures formed by several types of specialised cell. From prevention and treatment viewpoints, an improved understanding of normal tooth development is desirable. For example, enamel formed in the presence of fluoride is relatively resistant to dental decay but, excess fluoride can cause undesirable defects that commonly manifest as white spots in enamel. Better fundamental understanding of enamel formation could lead to more acceptable use of fluoride and to the development of alternative nutritional strategies that promote resistance to tooth decay. The ability to regulate odontoblast activities might also be translated into clinical benefits, such as improved repair processes after reimplantation of traumatically displaced teeth. Definition of dental tissue proteomes will provide stringent diagnostic markers for dental histopathology, and establish a comprehensive "fingerprint" for assessing authenticity of dental cell lines. Proteomics has great potential to underpin a comprehensive understanding of tooth formation, to the benefit of several scientific fields. Recently, there has been an increased interest in unravelling the cellular pathways controlling the differentiation and proliferation of human stem cell lines. Proteome analysis has proven to be an effective approach to comprehensive analysis of the regulatory network of differentiation. Also, proteomic map of abundantly expressed proteins in stromal cells derived from the dental pulp can be established. Differential protein expression profiling will provide a basis for elucidating the protein expression patterns and molecular cues that are crucial in specifying the characteristic growth and developmental capacity of dental and non-dental tissue-derived mesenchymal stem cells. These expression patterns can serve as important tools for the regeneration of particular tissues in future stem cell-based tissue engineering studies. This article outlines the proteomic approaches in dentistry, and reviews some findings that hold general biomedical consequence.

Keywords: Dental stem cell, Dental tissues, Proteomics.

P-8

Proteome Analysis of Tomato Leaves Under Salt Stress

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Salt stress is certainly one of the most serious environmental factors limiting the productivity of crop plants. The identification of candidate genes for marker-assisted selection (MAS) could greatly improve the efficiency of breeding for increased salt tolerance. Salt-induced changes in the proteome could highlight important genes because of its high resolution for protein separation by two-dimensional gel electrophoresis and identification by mass spectrometry and database search. Tomato (*Lycopersicon esculentum* mill.) is a model plant for studying the mechanisms of salt tolerance in plants. Seeds of tomato cultivar Shirazy were cultured in WA medium, and after germination, seedlings were transferred to MS medium supplemented with 0, 80 and 160 mM NaCl salt. After 24 days, leaf samples were collected for protein extraction and to measure shoot dry weight. Changes induced in leaf proteins were studied by one-dimensional SDS-PAGE and two-dimensional gel electrophoresis. With increasing in salt stress, shoot dry weight decreased. In SDS-PAGE, at least one protein with molecular weight about 32 kDa whose expression was specifically increased under salt stress was identified. On 2-DE gel, more than 400 protein spots reproducibly were detected. At least 18 spots showed significant changes under salt stress. Of these eighteen spots, three new leaf proteins are induced, six proteins showed up-regulated and five spots showed down-regulated by salt stress. In addition, salinity inhibits the synthesis of four leaf proteins. Ten protein spots were analyzed by matrix- assistant laser desorption/ionization-time of flight (MALDI-TOF), leading to identification of proteins that some of these proteins could contribute a physiological advantage under salt stress, making them potential targets for MAS.

Key words: Two dimensional electrophoresis, *Lycopersicon esculentum*, Salt stress, Proteome, SDS-PAGE.

P-9

Sample preparation of human serum for the analysis of psoriasis biomarkers

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Psoriasis is a chronic inflammatory skin disease and associated with a number of biochemical and immunological disturbances Proteomic technologies are being used to discover and identify disease-associated biomarkers. The application of these technologies in the search for biomarkers in the serum of patients has be limited by the presence of highly abundant albumin. The removal of human serum albumin prior to proteome analysis is favorable in the biomarker discovery process. In this study, two different depletion strategies for removing albumin from human serum, TCA/acetone precipitation and albumin deplete kit (Aurum serum protein mini kit, Bio-Rad) were evaluated for improving protein profiling pattern before two-dimensional gel electrophoresis (2-DE). Serum samples were treated with either TCA/acetone or Aurum kit and then subjected to 2-DE. Protein spots were visualized by staining with silver nitrate. Both strategies resulted in reliable albumin depletion but the pattern of protein spots with both treatment methods was not similar. Direct comparison between treatments revealed three protein spots (biomarker) to TCA/acetone while only one spot were unique after Aurum kit treatment. The results demonstrate that these two treatments can represent an efficient complementary method for biomarker discovery.

Keywords: Albumin depletion, biomarker, two-dimensional gel electrophoresis

P-10

Study of Protein Pattern of Nine Genotype of *Brassica napus* by Sodium Dodecyl Sulfate Polyacrylamid Gel Electrophoresis

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Rapeseed (*Brasica napus* L.) is one of the most important Oil crops in many countries including Iran. Rapeseed is one of the most important source of edible plant oil and animal feed protein, also an important bio-energy crop, increasingly becoming a major crop worldwide. Genetic diversity is one of the most important criteria to select parents in breeding program. The experiment was laid out as a randomized Complete Block Design (RCBD), In this study, 9 genotypes of *Brassica napus* were grown in the experimental field, in of agronomy and plant breeding department of agriculture faculty, Razi university of Kermanshah in 2008. genotypes in study includes Dante, ARC-2, ARC-5, SLM-046, Gernimo, Zarfam, Talent, Rainbow, Opera. For SDS-PAGE method using sample leaf rapeseed ago maturation stage. Total protein of the samples were extracted by extraction buffer (Tris-Hcl, 50 mM, pH 8.5 containing NP-40, 2%; PMSF, 1mM and EDTA,1 mM, MgCl2, 20 mM and 2- mercaptoethanol 2%) and resolved in 12.5% and 5% resolving and stacking gels, respectively. The results of SDS-PAGE showed that protein pattern of the genotypes are different of protein bands with molecular mass of 90 ,85, 20, 18 and 16 KDa.

Key Word: *Brassica napus*, SDS-PAGE, Genotypes, Leaf protein.

P-11

Evaluated of Wheat Varieties by Seed storage Protein electrophoresis

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Wheat (*Triticum aestivum* L.) seed-storage proteins represent an important source of food and energy, The variability of seed storage-proteins was analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). In this study Seed proteins profile of 2 cultivars *Triticum aestivum* include Sardari and Pishtaz were analysis by Sodium Dodecyl Sulfate polyacrylamid gel electrophoresis (SDS-PAGE). Total protein of the samples were extracted by extraction buffer (Tris-Hcl, 50 mM, pH 8.5 containing NP-40, 0.2%; PMSF, 1mM and EDTA,1 mM, MgCl2, 20 mM and 2- mercaptoethanol 2%) and resolved in 12.5% and 5% resolving and stacking gels, respectively. It is concluded that seed storage protein profiles could be useful markers in the studies of genetic diversity. The results of SDS-PAGE showed that protein pattern of the Varieties were different in protein bands with molecular mass of 78, 70, 54, 35, 23, 24 and 16 kDa.

Key Word: *Triticum aestivum*, SDS-PAGE, Seed protein.

P-12

A proteomics approach to decipher the signaling pathway in methyl jasmonate elicitated hairy roots of *Silybum marianum*

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Silymarin (SLM) is a secondary metabolite and antihepatotoxic polyphenolic substance isolated from the milk thistle plant, *Silybum marianum* L. Gaertn. Currently one of the most convenient and productive way for production of SLM is hairy root culture of transformed plant using *A. rhizogenes*. It has been shown that production of secondary metabolite can be induced by elicitation of the culture with suitable biotic and abiotic elicitors. Exogenously applied jasmonate and its derivatives enhance production of secondary metabolites in a variety of plant species. In this study, the hairy roots culture were supplemented with 100µM methyl jasmonate and then measured the content of SLM. We observed that the SLM content increased significantly after 48h of methyl jasmonate elicitation and remained constant during the next hours. To elucidate the global effect of jasmonate on gene expression of *S. marianum,* we employed two-dimensional gel electrophoresis coupled with tandem mass spectrometry. Out of more than 670 reproducible protein spots were analyzed on each given gel, 26 protein spots were up or down-regulated upon methyl jasmonate treatment. Of them, ten proteins could be identified by mass spectrometry such as Luminal binding protein, glutamine synthetase, pathogenesis-related protein, caffeoyl CoA O-methyltransferase and Profilin-1. The possible implications of these proteins in increasing SLM production in *S. marianum* hairy root cultures will be discussed.

Key words: Silymarin, Hairy root culture, Methyl jasmonate, Two-dimensional gel electrophoresis

P-13

Proteomic analysis of somatic embryogenesis in saffron (*Crocus sativus* L.)

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Somatic embryogenesis could be considered as an efficient vegetative propagation method in the economically important species *Crocus sativus* L.. Moreover, this *in vitro* regenerative pathway can also be used a suitable model system for related studies in plant developmental physiology. Two dimensional gel electrophoresis combined with matrix-assisted laser desorption ionization time-of flight mass spectrometry (MALDI-TOF/TOF MS) was employed to study the somatic embryogenesis (SE) in Iranian saffron (*C. sativus* L.) collected from Khorasan province. Corms harvested in August were used as explant to induce embryogenic (globular stage embryos) and non-embryogenic calli. Proteome of the original explants and that of the induced calli were compared. In another experiment, proteome of the mature embryos (torpedo stage) of the second treatment were compared with proteome of their original explants; corms collected in March. Spots showed significant differences at $P \leq 0.01$ using t_ student test. Among them, 36 spots with more than 2.5 or less than 0.4 fold change were selected and extracted from colloidal coomassie-stained two-dimensional electrophoresis gels over a *p*I nonlinear range of 3–10 in the first dimension and using homogeneous 11.5% polyacrylamide gels in the second dimension and were subjected to MALTD-TOF/TOF MS. Among the identified proteins, 27 spots had the score above 71 and were involved in protein folding, cell metabolism, reactive oxygen scavenging, citric acid cycle, defense and cell apoptosis prevention. Based on these findings, oxidative stress was implemented in enhancement of somatic embryogenesis. The presence of essential proteins of nitrogen metabolism, such as glutamine synthetase protein, was also observed. We concluded that the involvement of different proteins from different functional groups are essential in the induction and promotion of embryogenesis in *C. sativus*.

Key word: *Crocus sativus* L., somatic embryogenesis, two dimensional gel electrophoresis. MALDI-TOF/TOF.

P-14

Bioinformatics analysis of adipokines

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Adipose tissue plays a crucial role in the regulation of whole-body fatty acid homeostasis. During the last 10 years, adipose tissue has come into focus as an endocrine organ important for development of many diseases related to obesity including: insulin resistance, type 2 diabetes, dyslipidemia, hypertensionand cardiovascular disease. Adipose tissue secretes a variety of bioactive peptides that play important roles in insulin action, energy homeostasis, inflammation, and cell growth. These secretory proteins from the adipose organ are named adipokines and have many physiological effects on different organs including the brain, bone, reproductive organs, liver, skeletal muscles, immune cells and blood vessels. Adiponectin is secreted exclusively from adipose tissue and is an abundant plasma protein. Adipokines may locally regulate fat mass by modulating adipocyte size/number or angiogenesis and inversely increased fat mass leads to dysregulation of adipocyte functions. In this research we consider to analyze the sequences of different adipokines and multiple alignments of protein sequences in order to identify conserved sequence regions, possible similarities and differences and understanding of biological and evolutionary relationship between adipokins. In this regard the protein sequence of nine adipokines which are believed to be involved in fatty acid metabolism and obesity were extracted from UnipratKB/Swissprot entries in a FASTA format and the data were analyzed by employing Multiple Sequence Alignment using ClustalW program. Results revealed that leptin and visfatine have the most and RBP-4 and IL-6 have the least homology in sequence. According to the phylogeny tree the proteins with the minimum distance are leptin and visfatin. These results suggest that visfatin not only interacts with leptin but also with other adipokines investigated in this research.

P-15

A New method for recognizing Sequences Similarity by wavelet transform

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Protein comparison and alignment still represent one of the most important and greatly used methods of protein sequence analysis. Previous approaches such as FASTA, BLAST and PROSRCH are mainly based on sequence comparison and alignment. In this article is the wavelet transform (WT) representation. It is a signal processing method for multi-resolution analysis and protein local feature extraction. This new similarity concept is an sequence similarity approach, which only takes into account the pairwise amino acid match. In this article sequence similarity computing based on the discrete wavelet transform (DWT) and analysis of numerical series of protein sequences.
we can conclude that the wavelet transform method could be established as a novel approach to examine protein sequences similarity.

Keywords: protein Sequence **,** discrete transform ,sequence similarity ,sequence comparison

P-16

Formation of neurodegenerative disease agent, amyloid fibril, of protein α-lactalbumin in the environment resembling inside body

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Several sporadic and genetic diseases are caused by protein misfolding. These include cystic fibrosis and other devastating diseases of childhood as well as Alzheimer's, Parkinson's and other debilitating maladies of the elderly. These diseases, is the occurrence of protein aggregates in ordered fibrillar structures known as amyloid found inside and outside of brain cells. The appearance of aggregates in diseased brains implies an underlying incapacity in the cellular machinery of molecular chaperones that normally functions to prevent the accumulation of misfolded proteins.

α-Lactalbumin (α-LA) is a small (Mr 14 200), acidic (pI 4.5), Ca^{2+}. binding milk protein and containing four disulphide bonds, which adopts a partially folded conformation under denaturing conditions. At these conditions, α-lactalbumin forms the so-called 'A' state with characteristics of a molten globule state (i.e. most of the secondary structure is in place but little tertiary structure is present), which makes it prone to amyloid fibril formation.

In vitro, α-lactalbumin forms amyloid Fibril after reductions in the presence and absence of dextran (68 kDa) as a macromolecular crowding agent. Here, we report that α-casein, a molecular chaperone found in milk is a potent in vitro inhibitor of α-lactalbumin fibrillization. In doing so, large irregular aggregates of α-lactalbumin are formed. α-Casein acts as a molecular chaperone to prevent the stress-induced, amyloid fibril of target protein. Compared to absent of dextran, the chaperone activity of α-casein have decreased against the fibrillation of reduced α -lactalbumin. Interaction between the chaperone and α-lactalbumin and formation of a complex are indicated by intrinsic fluorescence intensity, ANS binding assay and size-exclusion chromatography. In summary, α-casein interacts with α -lactalbumin and prevents amyloid fibril formation but it is a poorer chaperon in the presence of dextran.

P-17

Bioinformatics investigation of 3D structure of growth hormone- growth hormone receptor complex

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Human growth hormone (hGH) is a proteohormone secreted by the pituitary gland it regulates somatic growth, substrate metabolism and body composition and Its actions are elaborated through the GH receptor (GHR). This receptor has 4 isoforms. Isoform 1 (GHRfl) has 638 amino acids. Isoforms 2 (GHRtr) and 3 consist of amino acids 1-279, 1-277 respectively. Isoform 4 (GHRd3) has 616 amino acids.Structural analysis of the 1:1 growth hormone-receptor complex reveals the molecular basis for receptor affinity. Therefore through bioinformatics literatures and websites we evaluated 3D structure of the 1:1 growth hormone-receptor complex. General information, description and origin, references of the hGHR could be found In the site (http://srs.ebi.ac.uk/srsbin/cgi-bin/wgetz?[swissprot-ID:GHR_HUMAN]+-e). The protein (hGHR) has 4 isoforms and 4 domains, belongs to the type I cytokine receptor family and contains 1 fibronectin type-III domain. We have extracted the graphical structural features of hGHR in the site (http://expasy.org/cgibin/ aligner?P10912). We have found feature aligner of the receptor (sequences of domains and motifs) by use of a Sequence Element Veiwer Version 2.0b. Crystallographic structure of the 1:1 complex between G120R mutants of human growth hormone (hGH) and the receptor extra cellular domain (hGHbp) have been determined. By using of PDB Protein workshop 1.50 (powered by the MBT), veiwerlite and PyMOL viewer softwares, we have elucidated the 3D picture of 1:1 complex of human growth hormone with hGHR extra cellular domain. We could not find growth hormone receptor complete crystallographic structure. We could only elucidate the 1:1 complex of human growth hormone with hGHR extra cellular domain and hGHR extra cellular domain by itself. In this paper we collected different views of the protein structure by using of the softwares.

P-18

A New Algorithm for computing Sequences Similarity

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Many algorithms have been developed to search protein sequences which are used various methodologies and protocols for computation of sequences similarity. The paper deals with Mobile Frame Pattern Algorithm which is designed and offered with dynamic programming in order to compute secondary structure of proteins. Unlike BLAST, an alignment search tool which its output is parsed hardly for finding sequences similarity, the proposed algorithm is a faster and more effective method alignment concept for finding similar sequences in proteins and nucleotides.

The proposed algorithm finds all possible similarities between two sequences through frame pattern detection and comparing patterns with each other and finally determines similarity percentage of sequencesthe time complexity $O(knm)(k \prec 0.75)$ for worst case, O(b *n) where b <6 for average case and O(1) for best case.

The algorithm was implemented in C# and its output was a detailed report. The program is accessible through a site, as well.

P-19

Is beta 2 glycoprotein I involvement in redox reactions a key to determine its physiological function?

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Beta 2 glycoprotein I (beta2GPI) is a glycosylated abundant protein in plasma which is evolutionary conserved among mammalian species. Beta2GPI is also recognised as a dominant antigenic target in antiphospholipid syndrome, a condition considered as the most common cause of acquired hypercoagulability and morbidity in pregnancy. Beta2GPI was known to possess an anticoagulant function which is inhibited by anti phospholipid auto antibodies. However, our group has recently reported different aspects of B2GPI in coagulation.

Analysis of the crystal structure of β2GPI predicted disulfide bonds with the potential to participate in reactions with thiol oxidoreductases. The current study was designed to determine whether β2GPI molecule can be involved in thiol exchange reactions in vivo.

Recombinant beta2GPI was expressed in baculovirus system and purified using Ni-NTA metal-affinity chromatography. Native beta2GPI was purified from human plasma using HPLC. Both recombinant and native beta 2 GPI were reduced by TRX-1, and free thiols were labeled by a biotin thiol specific probe (MPB).The labeled cysteine residue(s) were detected by mass spectrometry. This study shows, for the first time, that thioredoxin-1 (TRX-1) generates free thiols in β2GPI. The second novel finding of this work is the first time evidence for the natural occurrence of free thiols within beta2GPI *in vivo*. Multiple human and murine serum samples were incubated with MPB. Reduced beta2GPI was determined with ELISA method using streptavidin coated plates. The MPB labelled beta2GPI$^{-/-}$ mouse demonstrates a significantly reduced signal versus beta2GPI positive murine samples.

The physiological implication of beta2GPI involvement in thiol exchange reactions needs further delineation. This novel finding may further explain the dual function of beta2GPI *in vivo*.

P-20

Insight to the amyloid fibril structure by κ-casein in the presence of reducing agent and temperature

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Amyloid fibrils are aggregated and precipitated forms of protein in which the protein exists in highly ordered, long, unbranching threadlike formations that are stable and resistant to degradation by proteases. Amyloid fibrils have been identified in bovine, rat and canine mammary glands known as corpora mammary glands. The proteins involved in amyloid fibril formation have not been determined, however several milk proteins including the casein have been suggested. κ-Casein molecules interact strongly with each other and the proteins exist in solution as aggregates of about six to seven molecules. In this study κ-casein aggregation and amyloid fibril formation has been studied at temperatures between 25 and 48ºC, by ThT binding assay, Transmission electron microscopy (TEM) and HPLC. κ-Casein readily forms amyloid fibrils following reduction of its disulfide bonds which increase with increasing temperature. Our findings suggest that reducing agent and temperature both induces fibril formation of κ-casein possibly by raising the monomeric state of κ-casein and accelerating its interaction with other κ-casein molecules and eventually forming fibrils.

P-21

Comparative analysis of Intestinal microflora from Normal and Obese Wistar rat

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Background: In obesity, the amount of energy expenditure exceeds energy consumption by less than 1%. This small difference over time may result in obesity phenotype . Role of environmental factors is quite apparent in obesity progression. Gut ecosystem can be considered as an environmental factor. The structure of this ecosystem is regulating by many factors and therefore it is crucial to identify the constituents of the system for better understanding of the host symbiont relationships. To asses the intestinal microbial diversity in DNA samples from intestinal microflora from normal (control) and obese *wistar* rat, we choose rat as model organism

Methods: Study of normal (wild-type) and obese *wistar* rat intestinal microflora using 16S rRNA gene sequencing based on molecular technique (cloning). Through this study, we tried to elucidate the bacterial community structures diversity from normal and obese *wistar* rat using newly reported primer set (8FI/907RI)

Results: Comparison of the two libraries shows significant differences in genus level compositions in normal, obese intestinal *wistar* rat. Genus Peptostreptococcaceae Incertae Sedis and Allobaculum is significantly higher in control rat while genus Turicibacter is significantly higher in obese rat

Conclusion: Firmicutes are found dominant in control rat intestine as compared to obese rat.

P-22

Peptidomics Technologies and Applications in Drug Research

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At present, approximately 500 proteases have been identified in the human genome that are either involved in the degradation of proteins or liberate novel bioactive peptides. Peptides control important cellular and physiological functions and possess enormous potential in treating diabetes, multiple sclerosis and osteoporosis. Spurred by the use of insulin as an archetype therapeutic peptide, peptide formulation and delivery technologies have been improved in recent decades, and revenues from therapeutic peptides are therefore expected to increase tremendously in the future. Peptidomics is a novel approach to the comprehensive display and identification of dynamic changes in all endogenous peptides and small proteins (peptidome) in response to physiological or pathophysiological conditions, or to therapeutic interventions. The peptidomics technology platform integrates purification of peptides and small proteins (< 20 kDa) from complex protein mixtures by high-performance separation via HPLC, with mass spectrometry analysis of fractionated peptides. This approach is particularly useful for identifying novel peptide drug candidates and peptide biomarkers that are coupled to the molecular events associated with disease pathomechanisms. These biomarkers are increasingly used in all phases of the drug development process and provide a measure for drug safety, efficacy, toxicity and side effects. Combined with genomics and proteomics, peptidomics enables the identification and development of peptide biomarkers that, knowing the amino acid sequence, can be translated into clinical assays such as radioimmunoassays (RIA) or enzymelinked immunosorbent assays (ELISA).

Key Words: Proteomics , Peptidomics, Drug research, Biomarkers.

P-23

Structural alignment and structure-function relationships of some defined therapeutic monoclonal antibodies using antibody engineering approach

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For the most part, interaction of antibodies and their antigens is performed with high efficiency and specificity. This is an essential prerequisite to use them for the targeted treatment of diseases. Recently, an antibody is discovered which is able to attach to two separate antigens. This state is inconsistent with “the lock and key” hypothesis used about antibodies and this occurrence has two main reasons. First, this antibody, called dual specific bh1, has an extraordinary flexibility, which is essential to create separated conformation for joining to each antigen. Secondly, diverse amino acids are critical in connecting to each antigen.

This information made hypothesis about structural study by using some defined therapeutic monoclonal antibodies to search for a new dual specificity in them. This study was performed with the 3D structure of bevacizumab in complex with the VEGF, of cetuximab in complex with EGFR, of pertuzumab with HER2/neu and of trastuzumab in complex with ERBB2.

3D structural alignments of bevacizumab, cetuximab, pertuzumab and trastuzumab were performed with MUSTANG and ProBiS softwares. Hydrogen’s bonds, involved in antigen-antibody interaction, in each structure were obtained from IMGT/3Dstructure-DB and compared with each other. Most of the contact residues of bevacizumab with VEGF are located in the three CDR loops of the VH domain, particularly the VH CDR3. Cetuximab binds the domain III of the EGFR, mainly through its VH CDR2 and CDR3. Pertuzumab binds the domain II of ERBB2 through the three VH CDR and trastuzumab recognises three loops on domain IV of ERBB2, CDR3 of both VH and V-KAPPA seem to be involved in this interaction. Finally, AutoDock4.2 software was used for docking analysis of each antibody with the aim of achieving a dual specificity in them. For example, cetuximab was docked with erbB2 and VEGF antigens, but we didn’t meet any new specificity in these antibodies.

Key words: therapeutic monoclonal antibodies, structural study, dual specific

P-24

Different proteolytic activities of fig proteases(Ficin) on milk proteins

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Ficus family such as Fig latex contains a group of cysteine proteases of 25000 in size named Ficin (E.C.3.4.4.12).This plant protease is used in drug and food industries for production of digestive drugs,meat tenderization and cheese production.

To study the proteolytic activity of ficin on milk proteins (casein, alpha-lactalbumine and beta-lactoglubulin), ficin was separated from latex and fig fruit extraction by ion-exchange chromatography and activity of it was studied on milk proteins in different buffer conditions by a standard spectrophotometric method and SDS-PAGE.

The results showed that ficin has significant proteolytic activity on casein in all buffer conditions,partial activity on alpha-lactalbumine only in acidic buffer and on beta-lactoglubulin only in basic condition.This study showed special proteolytic activity of ficin on milk casein protein.

Keywords: Fig,Cysteine protease,Ficin,Milk Proteins,SDS-PAGE

P-25

Isolation and cloning of rice (*oryza sativa*) thioredoxin h-encoding genes

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Thioredoxins (Trxs) are ubiquitous, low-molecular-mass proteins that are characterized by the presence of an exposed active site with the amino acid sequence of WC(G/P)PC. Trxs participate in numerous redox processes via the reversible disulfide/dithiol reduction reaction involving active site cysteines. Plant cells have different types Trx isoforms. Trx f, m, x, and y are found in the chloroplast, Trx o is localized in mitochondria and Trx h is typically cytosolic. Trx h isoforms are reduced by NADPH via NADPH-dependent thioredoxin reductases (NTR). Despite widespread studies on NTR/Trx system in plants, there has been little known about the specific functions to individual Trx h isoforms. In rice (*Oryza sative*) genome database there are 30 potential Trx-protein encoding genes out of which nine encode h- type Trx isoforms. Therefore rice can be a good model plant for Trx system research. We aim to produce recombinant rice Trx h isofrorms in *Escherichia coli* and compare them based on their *invitro* disulfide reduction activity in parallel with analysis of their appearance pattern in different rice tissues. Here we describe the isolation and cloning of three full length cDNAs encoding OsTrxh isoforms (OsTrxh1, OsTrxh20 and OsTrxh23). This provides the primary genetic materials for production of recombinant isoforms that will enable us to analyze their *invitro* interaction with Trx h target proteins or NTR.

P-26

Cloning and expression of the Influenza virus M2-Hsp70 fusion protein in eukaryotic and prokaryotic systems and qualitative and quantitative comparison evaluation

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Because of high mutation rates in the viral genome segment encoding HA, influenza variant strains resistant to the vaccine-induced immune responses emerge continuously. Thus, influenza vaccines have to be updated annually in order to be effective against circulating viral strains. Therefore, novel influenza vaccine strategies targeting conserved viral antigens and capable of eliciting cross-strain protection are dearly needed. Due to its conservation, the extracellular domain of the influenza A M2 protein (M2e) has the potential for being applied as a recombinant vaccine candidate against a wide range of strains, though its immunogenicity may need to be improved. The occurrence of several post-translational modifications within the structure of M2 protein may affect its immunopotency for the induction of humoral immune response. Herein, to construct a recombinant M2e-based vaccine candidate with the appropriate structural conformation and immunogenicity the corresponding nucleotide sequence from an H9N2 influenza strain was fused to the N-terminus of the truncated Mycobacterium tuberculosis HSP70359–610, as a potent adjuvant, and following its cloning into the pPICZaA plasmid (for *Pichia pastoris* KM71H yeast) and pQE-60 plasmid (for *Escherichia coli* M-15) the fusion gene was successfully expressed in both of systems. The secreted protein was then easily purified from the culture media, based on the presence of polyhistidine tag. Finally be compared the expression of both systems for quantity and quality.

P-27

Proteome analysis of acid lime infected with *Candidatus* Phytoplasma aurantifolia

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Candidatus Phytoplasma aurantifolia the causative agent of the witches broom disease in acid lime (*Citrus aurantifolia* L.), is responsible for major tree losses in Southern of Iran and Oman. This pathogen is strictly biotrophic, thus completely dependent on living host cells for its survival. The molecular basis of compatibility and disease development in this system is poorly understood. We applied a proteomics approach to analyze the gene expression of acid lime infected by *Candidatus* Phytoplasma aurantifolia. Samples were collected from healthy and infected plants and their proteome were analyzed using a two dimensional gel electrophoresis coupled with mass spectrometry. Out of 800 leaf proteins reproducibly detected in 8 replicates using Melanie 4 software, 64 proteins were observed to show significant response to the disease. MALDI TOF-TOF analysis of these protein resulted in identification of 42 proteins including proteins involved in oxidative stress defense, photosynthesis, metabolisms, stress response as well as small heat shock proteins. Our results will provide insight into the molecular basis of the infection process and identify genes that could help to inhibit the pathogen.

P-28

PlantProtein Extraction forProteomics Research;Problems and Opportunities

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Basically protein extraction is the base of proteomics research. If the extraction is not well done, the plant protein extract possess impurities and include other metabolites than proteins;the next steps like protein assays, gel electrophoresis , sequencing will face some problems. As a matter of fact there are several methods to extract proteins in proteomics research but choosing a suitable protocol for extraction of a plant depends on the plant,its metabolites, what materials and equipment are available and finally what are the aims of extraction.As plants consist of many metabolites that can make conflict with proteomics assays the best way to have the best and most pure result for proteomics research is to eliminate the other metabolites during protein extraction with the most suitable protocol. Reaching to this goal is to study plants and their metabolites and choosing the best protocol that can extract proteins among all the other existing metabolites. Of course all metabolites of plants are not known but in respect to the overall study on the metabolites of a plant and its biochemical figures, a new method can be introduced to find the suitable protocol for protein extraction for the studied plant. In this article the authors compare several plant protein extractions in order to find the best protocol suggestion for extraction of recalcitrant plants while they have done the methods on oil palm mature leaves as a recalcitrant plant. Finally the article explain the problems and outcome achievements as opportunities of protein extraction for oil palm plant as a case of recalcitrant plants and extend these findings for other plants in proteomics studies.

P-29

Rice NADP/thioredoxin system

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An NADP/thioredoxin system, consisting of NADPH, NADP-thioredoxin reductase (NTR), and its thioredoxin, thioredoxin h (Trxh) plays a posttranslational regulatory role by reducing disulfide bonds in target proteins involved in different cellular process. In contrast to prokaryotic and mammalian cells, plants have a complex NTR/Trx system comprising several Trx h and NTR isoforms. Therefore, one important question to be addressed is whether there is specificity in the interactions between different NTR and Trxh isoforms. Through search in rice (*oryza sativa*) genome database we identified nine potential genes encoding Trxh and four potential genes encoding NTR. We aim to produce purified recombinant forms of rice NTR and Trxh isoforms which will enable us to analyze *invitro* interaction between different isoforms of NTR and Trxh from the same organism. Here we describe isolation and cloning of two full length cDNAs encoding NTR, OsNTR1 and OsNTR2, from rice. The polypeptide deduced from these cDNAs shows high similarity with plant cytoplasmic or mitochondrial type NTR (A/B NTR) containing an FAD- and NADP-binding domain and an active site. The isolation and cloning of these genes provide the primary genetic materials for production of purified recombinant forms and investigation of their interaction with different OsTrxh isoforms.

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P-30

Network Reverse Engineering From Microarray Data

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High-throughput technologies have allowed the simultaneous measurement of the concentrations of thousands of molecular species in a biological system, such as mRNA, microRNA, proteins and metabolites. Large-scale gene expression profiling generates data sets that are rich in observed features but poor in numbers of observations. The organization of gene-expression profile data into functionally meaningful genetic information has proven difficult and so far has fallen short of uncovering the intricate structure of cellular interactions.

Machine learning approaches offer the potential to systematically identify transcriptional regulatory interactions from a compendium of microarray expression profiles. There are generally four major groups for reconstructing regulatory networks from high-throughput data including optimization methods -such as Bayesian networks-, regression techniques, integrative bioinformatics approaches and statistical methods.

Here we develop an unsupervised network inference algorithm, probabilistic relevance network (PRN), which overcomes many limitations of the existing algorithms. PRN algorithm applies both characteristics of optimization methods and statistical ones. PRN algorithm is compared with the other current inference network algorithms such as relevance network, ARACNe, CLR on the reconstruction of synthetic biochemical network, the results show that PRN algorithm increases the precision of the other current ones. We will also discuss application of similar approaches for generating regulatory network based on proteomics data.

P-31

Formalin-fixed paraffin-embedded (FFPE) proteome analysis using gel-free and gel-based proteomics

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Formalin-fixed paraffin embedded (FFPE) tissue has recently gained interest as an alternative to fresh/frozen tissue for retrospective protein biomarker discovery. However, during the fixation process proteins undergo degradation and cross-linking, making conventional protein analysis technologies problematic.

This is the first study systematically validating different extraction and separation methods of FFPE proteins using different gel-free and gel-based approaches. Incubation of tissue sections at high temperature with a novel extraction buffer resulted in improved protein recovery. Protein separation by 1-DE followed by LC-ESI MS/MS analysis was the most effective approach to identify proteins, based on the number of peptides reliably identified. Interestingly, a number of peptides were identified in regions of the 1DE that did not correspond to their native molecular weights. This is an indication of the formation of protein-protein complexes by cross-linking, and to protein fragmentation due to prolonged sample storage.

This study will facilitate the development of future proteomic analysis of FFPE tissue and provide a tool for the validation in clinical archival samples of biomarkers of exposure, prognosis and disease.

P-32

Differentially expressed of secreted proteins of the phytopathogenic bacterium *Xanthomonas citri* subsp. *citri* in interaction with plant extract

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*: they have same contribution in this work

Xanthomonas citri subsp, citri is a phytopathogenic proteobacterium responsible for the citrus bacterial canker. Based on the host range of the bacterium, two pathotypes have so far determined *X. citri* subsp. *citri* pathotype A, which causes severe infection on all *citrus* varieties worldwide. More recently, a second pathotype from different areas of Asia and Iran has been designated as *X. citri* subsp. *citri* pathotype A*. These A* strains are genetically related to *X. citri* subsp. *citri*, but their host range is primarily restricted to Mexican lime (*Citrus. aurantifolia*) and they do not infect grapefruit.
Genetically, these two pathotypes have shown to be different. Some studies on A-type strains revealed differentially expressed proteins in presence of host plant extracts (*in vivo* and *in vitro* conditions). In present study, we initiated a proteomics analysis to create a reference map of an Iranian A*-type strain of *X. citri subsp, citri* secretome. After numerous experiments to define the appropriate growth medium and growth conditions, extracellular proteins were isolated from *X. citri subsp, citri* culture supernatant and resolved by SDS-PAGE and two-dimensional gel electrophoresis. In this paper we try to show results obtained by 2D-gel electrophoresis of induced bacterial cells in culture medium containing host plant leaf extract compared to MM1 medium without leaf extract. We are also trying to determine by MALDI-TOF-TOF mass spectrometry the differentiated expressed proteins to identify their putative function.

P-33

Protein profiling of nickel resistance sterptomyses by 2-dimensional electrophoresis

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Introduction

2-dimensional (2-D) electrophoresis is a powerful and widely used method for the analysis of complex protein mixtures extracted from cells, tissues, or other biological samples. This technique separates proteins according to two independent properties in two discrete steps. Despite alternative technologies that have emerged, 2-D electrophoresis is currently the only technique that can be routinely applied for parallel quantitative expression profiling of large sets of complex protein mixtures. Furthermore, it delivers a map of intact proteins that reflects changes in protein expression level, isoforms, or post-translational modifications. In this study we used this method to analysis of complex protein mixture of two nickel resistance sterptomyses strains (E13 a super resistance strain and E16 a high resistance strain).

Materials and methods

Two Streptomyces strains (E13 and E16) Witch able to grow on concentrations higher than 100 mmol/l nickel were culture and harvested from CSM medium with Ni. Then the bacterial palate washed and grind by liquid nitrogen. After that we select the supernatant and add Acetone/DTT for precipitation of protein. Then we did isoelectric focusing (IEF) electrophoresis by Strip method for bacterial Proteins. After IEF, strips Equibrated and SDS-PAGE were done. At the end the PAGEs were stained and analysis their spots.

Results

Our result shows that E13 and E16 sterptomyses strains protein profile have high similarity in protein profiling. In super resistance strain we have 7 over expression spot witch detected by software. Also in this strain we don't have 4 expressions in compared with high resistance strain. Also we have any alter in protein position in super resistance strains.

Discussion

2D electrophoresis is a reproducible method for detecting proteins in cells. Our study shows that we can use this method for protein expression evaluate and compare with each other.

Key words: 2-dimensional electrophoresis, nickel resistance, sterptomyses

P-34

Production and Purification of recombinant outer membrane protein ($PilQ_{406-770}$) of *Neisseria meningitides*

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Introduction

Neisseria meningitides is a major cause of bacterial septicemia and meningitides. While, conjugated polysaccharide vaccines are available against infections caused with Meningococcal serogroups A, C, Y and W135, since cross-reactivity of the serogroup B capsule with human tissue has hampered efforts to develop a reliable vaccine. PilQ outer membrane protein is an attractive vaccine candidate because it has a conserved sequence and it is expressed abundantly on the cell surface of most Meningococci.

Materials & Methods

In the present study, $PilQ_{406-770}$ gene cloned in pET28a expression vector and the recombinant protein (r $PilQ_{406-770}$) was over expressed. The 1095bp fragment of *PilQ* gene ($PilQ_{406-770}$) was amplified by PCR from genomic DNA isolated from *N. meningitides* serogroup B strain CSBPI, G-245 and then cloned into the pET28a expression vector. For recombinant PilQ production, the recombinant plasmids were transformed into *E.coli* BL21, overexpressed, and the recombinant proteins were purified. Cells were grown at 37 °C in LB broth until they reached OD_{600} 0.5, then protein overexpression was induced with1 mM IPTG. The His-tagged proteins were purified by immobilized metal affinity chromatography with Ni-NTA agarose (Qiagen), under native or denaturing conditions. Protein concentrations were determined by Bradford analysis and the purity was determined by SDS-PAGE and Coomassie blue staining.

Results & Conclusion

Cloning of $PilQ_{406-770}$ was confirmed by colony-PCR and enzymatic digestion. SDS-PAGE analysis showed that prospected recombinant $PilQ_{406-770}$ protein with molecular weight of 43Kd was over expressed and carefully purified with affinity chromatography with Ni-NTA agarose. Thus, a prokaryotic high-level expression system for $PilQ_{406-770}$ protein was successfully established.

Key words: Cloning, Expression, *Neisseria meningitidis*, $PilQ_{406-770}$ protein

P-35

Purification of Chitinase from Native *Enterobacter cloacae* B4A

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Chitinases are digestive enzymes that break down glycosidic bonds in chitin. Chitinase activity has been found with bacteria and streptomyces, plants, invertebrates, vertebrates and fungi. A great deal of interest has been generated on chitinase because of its applications in the biocontrol of plant pathogenic fungi, molting process of insects, mosquito control, single cell protein and mycolytic enzyme preparation.
The production of chitinolytic enzymes has been widely reported in bacteria and filamentous fungi. However, it could be of interest to find new sources of enzymes in order to display new specificities. The present investigation studied the purification of chitinase of native *Entrobactiacea.*The enzyme was purified by ammonium sulfate precipitation and ion-exchange chromatography. The purity and molecular masses of polygalacturonase were determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on a slab gel prepared with 12% (resolving gel) and 5% (stacking gel) acrylamide and had an apparent molecular mass of about 50 kDa, as shown by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. Its optimum activity was at pH 7.5 and 50°C, and the K_m and V_{max} values of this enzyme (for colloidal chitin) were 0.0031 mg/mL and 0.26 µmol/min, respectively.
The development of enzyme products often relies on screening a large number of organisms for an enzyme activity with a specific set of biochemical and biophysical characteristics that suits the targeted population.

P-36

HOMOLOGOUS BACTERIAL PROTEINS IN *NOCARDIA*: A PROTEOME STUDY OF *Nocardia farcinica* SD1828, *N. africana* SD910 and SD 925, *N. sp.* 1086, and *N. asteroides* N317

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Background: Infections caused by *Nocardia* species are infrequent but challenging to clinicians. In recent years many new pathogenic species have been described including *Nocardia africana*, a pulmonary pathogens (Hamid et al., 2001). Information about the disease and its etiological agent is still poor.

Objective: The present study aims to investigate all proteins expressed by the genome of *Nocardia africana*, *N. farcinica* and *N. asteroides* and their structures and functions compared to *Mycobacterium tuberculosis* by 2-dimensional polyacrylamide gel electrophoresis (2D PAGE) and mass spectrometry using MALDI system.

Materials and Methods: a comparative gel-based analysis of five *Nocardia* strains was conducted and the prominent proteins were assigned with MALDImicroMX instrument. Database queries were carried out using Mascot (MatrixScience Ltd., London, UK) in-house with species limitation on bacteria (Koltzscher et al., 2003).

Results: 2D-PAGE using pH strips 3-10 revealed that the soluble proteins were visible in a much smaller pI range. All strains exhibited similar protein distributions. Prominent spots were excised from the gels and assigned. The original data and spectra can be downloaded from the NoDaMS (http://ifg-izkf.uni-muenster.de/proteomik/nodamsa) database. As is observed in many proteomes, heat shock factors, chaperones and metabolic enzymes such as enolase dominate the visible proteome. A similarity analysis revealed that *Mycobacterium* and to a lesser extent *Rhodococcus* sequences are of high relevance for the investigated strains.

Conclusions: A comparative proteomic study on five nocardia strains was conducted for the first time. Relations among the proteomes were established and prominent proteins were assigned with MALDI-MS. For further improvements a DIGE (Differential Gel Electrophoresis) based approach is planned where effects due to gel-to-gel variations are minimized. A large body of MS data is available in the NoDaMS database which was created to share peak lists for future investigations using advanced protein databases.

Keywords: Mass spectrometry, *Nocardia*, proteomics, chaperones

P-37

In vivo substrate identification of peptidases via comparative peptidomics

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Proteolytic enzymes, both proteases and peptidases, are involved in many aspects of cell physiology and development such as breakdown of storage proteins during seed germination, protein remobilization upon senescence, removal of transit peptides during protein import to organelles, and recycling of damaged or misfolded proteins. These enzymes process their specific endogenous substrate(s) leading to short peptides or amino acids, which may in fact have important roles in other cellular processes such as signaling, defense and biogenesis. Although in Arabidopsis more than 650 of such enzymes have been predicted to be involved in different steps of protein hydrolysis in different parts of the cell, true cellular substrates have been demonstrated for only a few of these enzymes.
Here, a chloroplast-localized acylaminoacyl-peptidase (cAAP; At5g36210) was studied. The enzyme catalyzes the removal of a N^{α}-acetylated amino acid from short peptides enhancing plant survival under etiolated conditions. In order to ascertain the in vivo substrate(s) of cAAP, a comparative peptidomics approach was undertaken to determine the N-terminally modified short peptides that are absent from the wild-type peptide pool but present in the *caap* peptide pool given that the cAAP is most likely the sole peptidase of this kind within chloroplast. Stromal peptides extracted from intact chloroplasts of both wild-type plants and *caap* mutants were fractionated via liquid chromatography and unique peaks were apparent in *caap*. The peptides in these peaks were further separated on a NanoLC and sequenced via tandem mass spectrometry. Our findings indicate that alanine and proline are the main acetylated residues of peptides that accumulate in *caap*.

P-38

Comparison of protein enrichment strategies for 2-DE analysis of human plasma

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The wide dynamic range of proteins in human sera makes their proteome analysis a challenging task. The high-abundant proteins tend to mask the presence of low-abundant ones in protein profiling experiments. In this study, two different commercially available protein-partitioning kits were tested for their ability to lower the dynamic range of proteins for efficient 2-DE. Aurum Serum Protein Mini Kit (Biorad), using affinity chromatography, was compared with the ProteoMiner Kit (Biorad), where proteins compete for binding sites on bead-bound hexapeptide with different binding properties. The pre-fractionated protein samples were analyzed by 2-DE, which revealed outstanding differences in protein patterns. The removal of human serum albumin together with immunoglobulin G will significantly increase the detectable protein bands and spots in 1-DE and 2-DE, respectively. It seems that incomplete depletion of Albumin and IgG besides the presence of other high abundant proteins still remains a serious problem in Aurum serum depletion strategy. Great differences were observed in protein pattern compared with Aurum Serum results when plasma was used for pre-fractionation using ProteoMiner; while much better results were achieved by using serum as a sample for treatment by means of ProteoMiner. The results indicated increased resolution and improved intensity of low-abundant proteins in 2-DE gels of sera treated with ProteoMiner compared with 2-DE gels from plasma treated with Aurum serum and ProteoMiner.

Key words: Plasma, 2-DE, ProteoMiner.

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P-39

Coronin-1 expression changes in mice lymphocytes infected by different strains of rabies virus: sign of diverse peripheral immune response

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Coronin 1 is a leukocyte specific regulator of Ca^{2+}-dependent signaling which interacts with phospholipase C-γ1 and is critical for the generation of inositol-1,4,5-trisphosphate. Coronin 1 is essential for the function and survival of peripheral T lymphocytes through release of Ca^{2+} from intracellular stores. While exerting the same task in B lymphocytes, it is not essential for in vivo B cell signaling. In this study using 2-DE followed by MALDI TOF mass spectrometry, we showed a significant decrease in expression level of coronin1 in spleen lymphocytes of mice infected by attenuated strain of RV, while the lymphocytes of mice infected by SV strain of RV showed the same level of coronin1 compare to control group. This observation suggests a significant change in the TCR and BCR activation signaling pathway in response to different strains of the RV after 4 days of viral infection. According to previous studies, it could be speculated that severe decrease in coronin1 expression level in response to PV, could be critically diminished peripheral T cell activation level while B cell response is not seriously affected.

Key words: Coronin1, Rabies, 2-DE, Lymphocytes.

P-40

ESI-GEMMA is a novel technique to study oligomerization of ribonucleotide reductase

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Gas-phase Electrophoretic Mobility Macromolecule Analysis (GEMMA) is a relatively new mass spectrometry-related technique to determine the molecular mass of protein complexes. This technique has similarities to electrospray ionization (ESI) based mass spectrometry used in proteomics but is much more sensitive towards large protein complexes than mass spectrometry is. In GEMMA, there is a charge neutralizer that certifies that the proteins have +1 charge (or less).

In order to clarify how the class Ia *E. coli* ribonucelotide reductase (RNR) is regulated and what role large complexes have in this regulation, GEMMA was used.

The RNR is a key enzyme for the synthesis of the four DNA building blocks. Class Ia RNRs contain two subunits, denoted R1 (α) and R2 (β). These enzymes are regulated via two nucleotide-binding allosteric sites on the R1 subunit, termed the specificity and overall activity sites. The specificity site binds ATP, dATP, dTTP or dGTP and determines the substrate to be reduced, whereas the overall activity site binds dATP (inhibitor) or ATP. By using GEMMA and enzyme assays, we found that the *E. coli* class Ia RNR formed an inhibited $\alpha_4\beta_4$ complex in the presence of dATP and an active $\alpha_2\beta_2$ complex in the presence of ATP. Studies of a known *E. coli* R1 mutant (H59A) showed that deficient dATP inhibition correlated with reduced ability to form $\alpha_4\beta_4$ complexes. ATP could also induce the formation of a generally inhibited $\alpha_4\beta_4$ complex in the *E. coli* RNR but only when used in combination with high concentrations of the specificity site effectors, dTTP/dGTP. Therefore, both allosteric sites are important for $\alpha_4\beta_4$ formation and overall activity regulation. The *E. coli* RNR differs from the mammalian enzyme which is stimulated by ATP also in combination with dGTP/dTTP, and forms active and inactive $\alpha_6\beta_2$ complexes.

P-41

Biological analysis and proteome mapping of *Hemiscorpius lepturus* venum

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Envenomation by *Hemiscorpius lepturus* is associated with local necrosis, followed by systemic manifestations including intravascular haemolysis, coagulopathy and acute renal failure/damage. Little is known about the mechanisms by which *H. lepturus* venom exerts these noxious effects. In an attempt to characterize the *H. lepturus* venom, collected from Khuzestan province, gelatin SDS-PAGE was employed which revealed the high molecular weight proteins with gelatinase activities. Moreover, in a rp-HPLC based fractionation strategy we found low molecular weight peptides showed neurotoxic activities, and a 32 kDa protein (as a single band in SDS-PAGE) showed highly hemolytic/dermonecrotic activities similar to Sphingomyelinase D from reported *Loxoscels recluse* and *L. intermedia*. A dose–response curve was plotted for phospholipase A2 (PLA2) activity in crude HL extract, using egg lecithin (phosphatidylcholin) as substrate. This activity was augmented prominently by increasing $CaCl_2$ concentration in medium. PLA2 activity in crude HL extract was inhibited 95% by 2.0 mM Na2–EDTA. For protein mapping of the venom we used 2-DE analysis which resolved 96 protein spots, with Mr of approximately 5-120 kDa; very high variation among other reported venoms.

P-42

Proteomics and metabolomics analysis of wheat stem reveals, improved stem reserve remobilization as a result of good coordination of enhanced stem senescence and oxidative stress defense under drought stress

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Drought is one of the major factors limiting the yield of wheat (*Triticum aestivum* L.) particularly during grain filling. Under terminal drought condition, remobilization of pre-stored carbohydrates in wheat stem to grain has a major contribution in yield. To determine the molecular mechanism of stem reserve utilization under drought condition, we compared stem proteome patterns and some metabolites of two contrasting wheat genotypes (N49 and N14) under a progressive post-anthesis drought stress, during which period N49 peduncle showed remarkably higher stem reserves remobilization efficiency compared to N14. Out of 830 protein spots reproducibly detected and analyzed on two dimensional electrophoresis gels, 135 spots showed significant changes in at least one genotype. The response was more pronounced in N49 compared to N14. The highest number of differentially expressed proteins was observed in genotype N49 at 20 days after anthesis when active remobilization of dry matter was observed, suggesting a possible involvement of theses proteins in effective stem reserve remobilization of N49. Identification of 96 of differentially expressed proteins using mass spectrometry revealed a coordinated expression of proteins involved in leaf senescence, oxidative stress defense, signal transduction, metabolisms and photosynthesis which might enable N49 to efficiently remobilized its stem reserves compared to N14. The up-regulation of several senescence-associated proteins and breakdown of photosynthetic proteins in N49 might reflect the fact that N49 increased carbon remobilization from the stem to the grains by enhancing senescence. Furthermore, the up-regulation of several oxidative stress defense proteins in N49 might suggest a more effective protection against oxidative stress during senescence in order to protect stem cells from premature cell death. Among metabolites, the results of amino acids: Methionine and ACC (1-aminocyclopropane-1-carboxylic acid) confirmed up-regulation of S-adenosylmethionine (SAM) synthetase to increases senescence by ethylene production. Our results suggest that wheat plant might response to soil drying by efficiently remobilize assimilates from stem to grain through coordinated gene expression.

Keywords: drought, stem, stem reserves, remobilization, two-dimensional electrophoresis, wheat, oxidative stress, senescence, grain filling, MALDI-TOF, MALDI-TOF/TOF, Q-TOF

P-43

Proteomics study of *Plasmodium falciparum* after chloroquine treatment

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The resistance of the malaria parasite *Plasmodium falciparum* to chloroquine represents an emerging problem since neither mode of drug action nor mechanisms of resistance are fully elucidated. We studied comparative level of protein expression, using 2DE-MS approach, across the parasite's intraerythrocytic stages in untreated and transiently IC_{50} chloroquine-treated cultures of the chloroquine-resistant (K1) and -sensitive (F32) strains of *P. falciparum*. Functional plasmodial protein groups found to be affected by chloroquine were among those central to the parasite's physiological processes, including protein folding, DNA repair, signal transduction and some host proteins. Here, we report some proteins like biliverdin reductase B and thiol-specific antioxidant protein involved in redox reactions like heme metabolism that were related to mechanism of chloroquine action.

Keywords: *Plasmodium falciparum*, Chloroquine, Drug resistant, Proteomics

P-44

The Study of Proteomic Responses of a Tolerant Cultivar of Barley to Salinity

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Responses of plants to salinity stress and the development of salt tolerance are extremely complex and various mechanisms appear to be involved. We employed a proteomic approach to further understand the mechanism of plant responses to salinity in a tolerant cultivar of barley (Afzal). Three-week-old seedlings were treated with 300 mM NaCl for 3 weeks. Total proteins of fourth leaf were extracted and separated by two-dimensional gel electrophoresis. 315 protein spots were reproducibly detected, including 68 that were up-regulated and 24 down-regulated. Using MALDI-TOF-TOF MS, we identified 25 proteins involved in many cellular functions. This proteins include Oxygen-evolving enhancer protein, Rubisco, putative glycine decarboxylase, ribosomal protein P1, ribosomal protein L12 homology, Nascent polypeptide-associated complex subunit alpha-like protein 3, chloroplast RNA-binding proteins, Translationally-controlled tumor protein homology, nucleoside diphosphate kinase, polyamine oxidase, oxalate oxidase-like protein or germin-like protein, Subtilisin-chymotrypsin inhibitor CI-1B and Ribulose bisphosphate carboxylase/oxygenase activase.

Keywords: Two-dimensional electrophoresis, Proteomics, Salinity, Barley

P-45

A comparative study of protein expression profile of fibrosarcoma cells transfected with p16INK4a

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The tumor suppressor p16INK4A regulates the cell cycle by inhibiting the cyclin D-dependent kinase CDK4/6 complex, thereby preventing phosphorylation of retinoblastoma (pRB) proteins which results in arresting the cells in the G_1 phase.
Inactivation of p16 has been occurred in a wide variety of malignant human tumors.
It is structurally comprised of four contiguous ankyrin repeats, which are believed to be involved in CDK4/6 interaction. Previous attempts regarding *in silico* and experimental assessments have revealed the minimum stable and functionally active domain of p16, to be the C-terminal half, named truncated structure G. This fragment was found to be able to induce G_1-cell cycle arrest in HT-1080 fibrosarcoma cells (negative for p16 expression), inhibit proliferation and physically interact with CDK4/6 via immunoprecipitaion analysis comparable to p16 wild type. To evaluate the effect of the ectopic expression of p16 together with this truncated form on the molecular pathway alterations in the proteomic profiling in HT-1080 fibrosarcoma cells, we performed 2-DE based proteomics analysis. The preliminary observations show significant changes in the protein expression profile among the experimental groups.

Keywords: p16INK4a, truncated, 2DE

P-46

γ-turn types prediction in protein using the two-stage hybrid neural discriminant model

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Due to the slightly success of protein secondary structure prediction using the various algorithmic and non algorithmic techniques, similar techniques have been developed for predicting γ-turns in proteins by Kaur and Raghava. However, the major limitation of previous methods was inability in predicting γ-turn types. In a recent investigation we introduced a sequence based predictor model for predicting γ-turn types in proteins. In the present work, in order to analyze the effect of sequence and structure in the formation of γ_turn types and predicting γ-turn types in proteins, we applied novel hybrid neural discriminant modeling procedure. As the result, this study clarified the efficiency of using the statistical model preprocessors in determining the effective parameters. Moreover, the optimal structure of neural network can be simplified by a preprocessor in the first stage of hybrid approach, thereby reducing the needed time for neural network training procedure in the second stage and the probability of over fitting occurrence decreased and a high precision and reliability obtained in this way.

P-47

Leave proteome analysis of two bread wheat cultivars (*Triticum aestivum* .L) under salinity stress

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Soil salinity is one of the limiting factors that adversely affects crop productivity and quality. Plants respond to this abiotic stress by activating the expression of stress-responsive genes. Proteomics approach based on two-dimensional gel electrophoresis, is a powerful method to investigate the changes in proteins expression when a plant has been exposed to salinity stress. In present trial we used two bread wheat (*Triticum aestivum* L.) cultivars to analyze changes in their protein expression at short time salinity stress. Karchia is a resistant cultivar but Gaspard is relatively a sensitive cultivar compared to Karchia. These two cultivars were planted in pots under greenhouse conditions. Pots were irrigated with 1/2 Hogland nutrient solution until seedling stage. At this growth stage, plants were then exposed to 100 and 200 mM NaCl solutions. The samplings were done after 12 and 24 h of stress treatment by removing seedling leaves. Total proteins were extracted using a marginally modified Damerval (1986) protocol. Proteins extracted from wheat leaves were separated by IPG gel pH range 4-7 in first dimension. In second dimension, polyacrilamide gel (SDS-PAGE) with concentration 12.5% was used. The results of second dimension gels analysis showed 125 protein spots that were significantly different from the control treatment in both wheat cultivars. Eight out of 125 spots were in concordance with bread wheat leaf proteome pattern as determined by their p*I* and MW. Comparing the percent volume of these 8 spots in Karchia & Gaspard cultivars showed that there are similar trend in up- and down- expressions of these proteins. Both photorespiration and signal transduction pathways were induced and superoxide dismutase enzyme increased in two cultivars under stress conditions.
Keywords: bread wheat; salinity stress; proteomics; two-dimensional gel electrophoresis.

P-48

Comparison of three refolding methods for purified recombinant streptokinase

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Introduction:

E.coli is widely and easily used for the high-yield expression of recombinant pharmaceutical proteins, however, a major drawback in this system is the refolding of the denatured proteins purified by solubilization of highly expressed inclusion bodies. Herein, we compared the effect of urea removal by three dialysis-based refolding methods on the activity of highly expressed and purified streptokinase, which is a thrombolytic serine protease.

Methods:

skg gene from GGS-S88 streptococcus strain was cloned, expressed in *E. coli* and following the solubilization of inclusion bodies by 8M urea, was purified based on a 6xHis-tag affinity chromatography. Refolding process was performed by dialysis either in reducing concentration gradient buffer of urea, or in 50 mM Mn^{+2} buffer or on the PEG20000 salt. Refolded proteins were assayed for enzyme activity by calorimetric test.

Results and conclusion:

Proteins refolded in all three methods were more active than non-refolded protein with the specific activities of 835.5, 3962.28 and 16512.66 U/mg for Mn^{+2} solution, PEG and urea gradient buffer, respectively. Although PEG removed the urea immediately and increased the concentration, protein was unable to recover its proper folding, may be due to the rapid acting. Mn^{+2} ions removed the urea efficiently, but they seemed to interfere with the SK active site or influence the substrate-SK interaction. However, the optimum process corresponded to the gradual activity recovering during 8 h of incubation in lowering concentration gradient of urea.

P-49

Preliminary results of serum proteomics analysis in search of potential *H.pylori* related gastric cancer

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Gastric cancer is the second fatal malignancy in the world and the fourth common cancer in Iran.

The association between *H.pylori* and gastric cancer is well established and the bacterium has been recognized as a class one carcinogen. The aim of the present study is to identify the potential serum biomarkers associated with *H. Pylori* related gastric cancer. We used a serum partitioning strategy, based on hexapeptide binding affinity, to increase the detection power of serum low abundant proteins. A comparative proteomics approach was applied to identify the differentially expressed proteins in different groups of pooled sera from *H.pylori* positive/negative patients with/without gastric cancer. 2-DE was used to separate the total proteins of serum in patient and related controls. After Silver nitrate staining and image analyzing, the differentially expressed proteins were determined between groups. Seven spots were found in *H.pylori* positive patients with gastric cancer showed significant expression level compare to *H.pylori* positive patients with no gastric cancer (group1). Meanwhile, 15 differentially expressed proteins were observed in sera of *H.pylori* negative patients with gastric cancer versus *H.pylori* negative healthy controls (group2). There were 27 spots with different expressional level in *H.pylori* positive against *H.pylori* negative healthy controls (group3).The comparison of the detected spots between these three groups revealed at least 5 candidate proteins as potential biomarkers for further investigation.

Keywords: Gastric cancer, proteomics, proteominer, serum biomarker

P-50

Functional Genomics of a Mesophilic Chitinase Isolated from Shrimp Pond

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Chitinase has potential applications in the production of pharmaceutically important chitooligosaccharides and N-acetyl D-glucosamine, treatment of chitinous waste and preparation of single-cell protein, isolation of protoplasts from fungi and yeast, control of plant pathogenic fungi and insects. However, it could be of interest to find new sources of enzymes in order to display new specificities. The present study focused on cloning and expression of chitinase gene from a highly chitinolytic local isolate of *Enterobacter cloacae* B4A in *Escherichia coli* BL21 (DE3) and comparison of the characteristics of the native and recombinant chitinases.
Genomic DNA of *Enterobacter* sp was isolated by DNA isolation Kit (Cinnagene). By using PCR technology, chitinase gene cloned in a recombinant plasmid. For this propose we were used modified primers carrying restriction sites for *NcoI* and *BglII*. The amplified gene products and pQE60 expression vector were digested with *NcoI* and *BglII*. An aliquot of ligation mixture was electroporated into competent cells of *E.coli* BL21. The clones were streaked onto LB agar plate containing ampicillin followed by its incubation at 37°C in a shaking incubator. 1 ml of overnight culture (OD=0.6) was used to induce with IPTG at 25°C and was used from samples for SDS-PAGE. The deduced amino acid sequence of chitinase has high degree of homology with chitinase from *Serratia marcescens*. The recombinant chitinase was purified to near homogeneity using His-Tag affinity chromatography. Further work on the scale-up production and application of this enzyme, and its economic/commercial feasibility is currently underway.

P-51

Study of changes in *Staphylococcus aureus* under salt stress by 2-DGE

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Investigation and characterization of proteins that involve in salt resistance process in prokaryotes are important as two aspects: microbial physiology and transferring genes are responsible for these proteins in order to molecular plant breeding. Proteomics method based on 2-DGE is powerful tool for analysis of changes resulted of gene expression in different biological conditions.

In this research, *Staphylococcus aureus* was used as a salt resistant bacterium. These bacteria were grown in two medium that only differed in NaCl concentration (2M, 0.2M). After 16h growth, protein extraction was performed by RNA-Xplus. The results of second dimension gels analysis with Melanie 6.02 indicated that 109 proteins showed significant response to the treatments.

Key words: *Staphylococcus aureus*, 2-DGE, salt stress

P-52

Stress- Induced Proteomics Changes in the Hippocampus of pregnant Wistar rats

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Stress is a threatening factor that all living creatures are encountered throughout life. Depending on the type of stress, there is several mechanisms for keeping body homeostasis to minimize stress effects. One of the early responses to stress by mammalian systems is secretion of corticosterone hormone. However, in highly stressful conditions, the body protective mechanisms fail and damage to the sensitive organs occurs. . Brain is an organ which shows high sensitivity to stress conditions, especially hippocampus that has an important role in memory creation process. There are a few limited studies reporting stress-induced changes in brain during pregnancy. To better understand the molecular pathways and role of molecules involved in stress-induced mediated brain effects, recent powerful techniques such as proteomics has recently become more available. In proteomics studies expressional differences of thousands of cellular proteins in different states can be analyzed. In the present study, immobilization stress in regular sessions was applied to a group of 6 pregnant Wistar rats and similar number of animals was used as control group receiving no stress. To verify stress induction, the quantity of corticosterone hormone in plasma samples was measured using ELISA test. Analysis of hormone results showed increaseed amount of corticosterone hormone in stress-induced pregnant rats. On the last day of pregnancy, rat hippocampus from the brain of animals in both groups was removed. Protein profiles of these samples were acquired using 2D Electrophoresis (2DE) technique and silver nitrate staining. Brain protein patterns on 2DE were compared using Image Master Software. In this analysis, 2476 proteins were detected and expressional differences were observed for 9 proteins. Among the nine proteins, 5 proteins were overexpressed while 3 proteins were underexpressed and one protein was totally absent in the stress-induced brain. Mass spectrometry identification of these proteins allows more precise analysis of stress-induced effects on protein expression during pregnancy.

Keywords: proteomics, stress, brain, pregnancy

P-53

Proteome Analysis of Rice Anther under Salt Stress

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Salinity is a major factor limiting rice production worldwide. Rice is generally considered to be sensitive to salinity during reproductive stage. Salt tolerance is a complex trait which can be studied from different physiological and molecular aspects. To determine the molecular mechanism of salinity tolerance at reproductive stage, we compared anther proteome patterns of two contrasting rice genotypes IR64 (sensitive) and Cheriviruppu (tolerant) under salinity stress. Plants were grown in IRRI green house and salt stress (100 mM NaCl) imposed at booting stage. Anther samples were collected from control and salt stressed plants at anthesis. Na/K ratio of IR64 genotype was more than 1.7 times under salinity as compared control condition but no significant change was observed in tolerant genotype (Cheriviruppu). Comparison of pollen viability for the mentioned genotypes showed 100% reduction due to 100mM NaCl for IR64 while this reduction in viability of pollen was 26% for Cherivirruppu (tolerant). showed Out of 454 protein spots reproducibly detected and analyzed on two dimensional electrophoresis gels, 18 stress responsive proteins could be identified by mass spectrometry (MALDI TOF/TOF) analysis resulting in identification of more than 18 proteins involved in several processes several salt responsive mechanisms which may increase plant adaptation to salt stress including, up-regulation of three isoforms of Fructokinase-2 in Cheriviruppu genotype under salt stress which play a role in regulation of pollen germination. The identification of stress responsive proteins of anther revealed several possible candidate mechanisms under salinity which may confer salinity tolerance.

Keywords: Anther, Proteomics, Rice, Salinity

P-54

Plasma protein analysis in patients with chronic hepatitis B infection

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Background: Hepatitis B virus initiates complicated cascade process leading to chronic hepatitis B (CHB), cirrhosis and hepatocellular carcinoma. The pattern of serum proteins and endogenous lipoproteins synthesized by liver could be changed during progressive liver disease. Proteomics analysis provides a specific and suitable alternative laboratory approach for the progression of liver disease.

Patients and methods: Twenty-five male patients with the diagnosis of CHB and five healthy controls were participated or evaluated in this study. Proteomic analysis of plasma proteins was performed by 2DE and mass spectrometry

Results: We found at least seven proteins that were changed significantly in HBV infected sera. These greatly altered proteins were identified as haptoglobin α and β chain, apolipoprotein A-1, α1-antitrypsin, transferring, transthyretin and ceruloplasmin. Apolipoprotein A-1 was significantly down regulated in serum samples of patients with advanced liver disease accompanied by lower expression of high density lipoprotein.

Conclusions: Evaluation of plasma proteins and apolipoprotein A-1 could be a useful serum biomarkers for the diagnosis of progressive liver disease in CHB. We concluded that impaired hepatic apolipoprotein synthesis may associate with advanced liver disease.

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P-55

Proteomics of Early and Late Cold Shock Stress on Thermophilic Bacterium, *Thermus sp. GH5*

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Thermus sp GH5 is an aerobic thermophilic bacterium. Its optimal growth is 70 -75 ºC and it was isolated from a hot spring in Ardabil, North West province of Iran. Due to industrial and biotechnological applications of thermophils, there is a vital need to know more about their proteomes and metabolomes. Since thermophiles live in stressful environments it will be very useful to study their survival mechanisms. There are many reports on stress induced proteins, particularly the well characterized heat shock proteins, but little is known about the functions of proteins induced after a decrease in temperature. In this study, the proteomes of the bacterium after a temperature downshift from 75 ˚C to 45 ˚C for 2h and 5h were investigated. We compared protein profiles of early and late cold shock processes to that of cells grown at 75°C and identified a set of proteins, some of which are involved in metabolism processes such as fatty acid synthesis, pentose phosphate pathway, aromatic component degradation and signal transduction. We identified the novel proteins that might be induced under cold shock conditions of *Thermus GH5***.** Our data showed this organism could be tolerating the stress conditions by changing in its metabolism and physiology.
Key word: *Thermus GH5*, thermophils, cold shock, stress, proteomics.

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